Plant Physiology
By
Shri. Ranjan.

PREFACE TO THE SECOND EDITION

In the preparation of this edition, certain topics that were over-looked in the first edition have been added while some others have been enlarged. At the end Appendix B has been added dealing with H-ion concentration. An attempt has also been made, keeping the limits of size set, to include the investigations and findings that have been made since the publication of the first edition.

My sincere thanks are offered to Dr. A .P. Mehrotra, and Mr. N. S. Parihar, M.Sc., Lecturers in Botany, Allahabad University, for their valuable aid in editing the manuscript.

120/B

DEPARTMENT OF BOTANY
INCLUDING AGRICULTURE,
ALLAHABAD UNIVERSITY.
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SHRI RANJAN.

PREFACE TO THE FIRST EDITION

This book has been written in response to a demand from the B.Sc. students of Botany of the University of Allahabad for a suitable text-book on plant physiology. The students in India have been very much handicapped in their study of this important branch of Botany for want of a suitable text-book: for, the standard text-books available are either too advanced for an average B.Sc. student or too elementary. In this book, therefore,—with my eyes on the student community, who, though not beginners are yet not sufficiently advanced,—I have endeavoured, so far as practicable, to mention the names of only a few important writers.

Nearly ten years ago in my leisure moments I sketched out a rough syllabus based upon my lectures on the subject. But for want of time due to various other commitments the work remained in an unfinished state till now. Some of the chapters of the book are based on the tripos Part I lectures on physiology delivered by Professor F. F. Blackman of the Botany School, Cambridge, while the chapters on the ascent of sap, growth and movements are compilations with suitable modifications from various text-books. An average B.Sc. student of an Indian University taking up Botany is generally ignorant of the elementary principles of logarithms. An elementary account of logarithms, therefore, is given as an appendix at the end of the

book. At the end of each chapter model practical experiments are also given which should prove useful to both teachers and students alike.

In the end I wish to express my thanks for the invaluable help that I received in the preparation of the figures and the revision of the manuscript from Dr. N. L. Pal and Dr. U. N. Chatterji—two of my former research pupils.

ALLAHABAD
December, 1944.

SHRI RANJAN.

INTRODUCTION

Probably from the time that Adam was banished from paradise, flowers have interested mankind. It is told in the scriptures that Adam was thrown out with only a flower from the garden of paradise. It had no magic charm as it was not the fruit, yet one can almost picture the emotions of the first man as he gazed at that lovely souvenir of paradise.

To some a flower is a mere blossom to be offered to the gods, to others it is a thing to be admired for its beauty and colour, but to a scientist it is a thing to be investigated and explored. It has been the endeavour of scientists, through the ages, to study in minute details the complex organisms that constitute life.

With the rapid growth of knowledge, the scientific study of matter around us was, for the sake of convenience, divided into distinct sub-divisions. Thus the study of plant life constitutes *Botany*. As knowledge of the subject advanced new names cropped up, *e.g.*, (*a*) *morphology* which deals with the investigation of forms and structures and (*b*) *physiology* which deals with the investigation of habit and functions. In the study of botany the morphologists ask 'What is this?' and in answer they analyse and anatomise the dead. The physiologists ask 'How is this?' and in answer they analyse the living.

The riddle of life is read, at present, in terms of physico-chemical changes associated with the living matter. Thus it will be assumed that the student of

physiology possesses a basic knowledge of physics and chemistry. The French philosopher Descartés has said "If, therefore, any one wishes to search out the truth of things in serious earnest, he ought not to select one special science; for all the sciences are conjoined with each other and interdependent." But physiology at present cannot explain everything in terms of the above. We have, therefore, to take recourse to such mystic words as "vital force." To a materialistic viewpoint, however, the action of vital force is akin to that of a catalyst influencing the rate of reaction.

The followers of vitalism, of course, believe that the conception of life cannot be found in physico-chemical researches, but in the researches which will expose the vitalistic phenomena. Vitalism is based on mysticism which unfortunately obstructs the progress of experimentation and observation on the principles on which modern science is based. One must realise, however, that to reach the elemental truths is not a matter of moments but the researches of centuries. It is said, "Truth is the daughter of time."

Green plants not only furnish us with all our foods; they also supply many of the basic materials of our industries. Success in plant cultivation has, therefore, become of utmost importance to our civilization. This success can only be attained by a proper understanding of the processes, and control over the activities, going on in the plant. Whether a person is a forester, or an orchardman, or an agriculturist or a floriculturist, he has ultimately to deal with the

fundamental problem of controlling some of the life processes in the plants for the advancement of his interests.

The aim of physiology is to endeavour to bring about the maximum plant production. For this, one has to study the reactions of the plant to the complex external and internal stimuli. The plant is but a result of the compounding of the internal stimuli with the external. The study of physiology is thus of supreme importance to agriculture.

The Unit of Life.

Properties of atoms depend upon the configuration and orderly arrangement of protons and electrons. So are also the properties of the living matter dependent upon the orderly arrangement of the cell-unit. The *unit* of the living matter is the *cell.* Inside the cell is the living matter, which was named by the Bohemian physiologist Purkinjé (1839) as *protoplasm,* the word meaning primitive form. In this the diverse physico-chemical changes go on and it is, therefore, the physical basis of life. A single-celled individual acts independently in a particular way. But in multicellular bodies, the independence of individual cells is merged into that of the entire organism, in which groups of cells work in unison, forming tissues. Yet, in these, the cells comprising such tissues retain, to a certain extent, their individuality. Therefore, the book aptly opens with the physiological study of a single cell.

CHAPTER I

MORPHOLOGY AND PHYSICO-CHEMICAL PROPERTIES OF THE CELL

Morphology of the Cell

Since a cell is the unit of structure and function it is essential that its morphological make-up be considered before its study from the physiological standpoint is taken up.

The cell-wall.—A plant cell is a minute speck of protoplasm of any shape bounded on the outside by a definite layer, the cell wall, which in young cells is almost entirely composed of cellulose. As the cell matures, the wall may get impregnated by other substances like lignin, suberin etc.

The protoplast.—The protoplasm inside the wall—protoplast is the name given to it—is demarcated into *cytoplasm* in which is embedded a specialised cell-organ, the *nucleus.*

The vacuole.—The cytoplasm and the nucleus together fill up the entire space within the wall in the younger condition of the cell. But, as the cell develops, spaces appear in the cytoplasm; these spaces or vacuoles are filled with a watery fluid having various substances dissolved in it and known as vacuolar sap or simply cell-sap. Later towards the maturity of the cell, these smaller vacuoles coalesce together forming one large vacuole centrally situated.

The membranes.—The cytoplasm, together with the nucleus embedded in it, then becomes necessarily

limited to a thin layer just inside the cell-wall enclosing the central vacuole. The outermost limiting layer of the cytoplasm just abutting on the cell-wall is somewhat differentiated from the rest of the cytoplasm inside. There is a similar differentiation of the innermost limiting layer of the cytoplasm next to the vacuole. Of these limiting membranes, the one next to the cell-wall is known as the external plasma

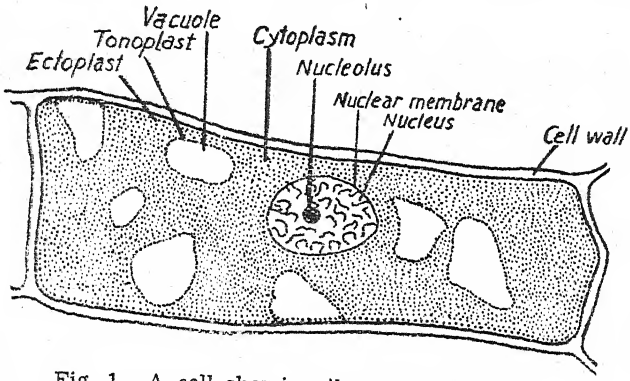


Fig. 1.—A cell showing the essential parts.

membrane or the *ectoplast* and that bounding the vacuole as the *tonoplast*. As a matter of fact boundary membranes are differentiated wherever within the cell two different surfaces come in contact.

The cytoplasmic inclusions.—There are differentiated in the cytoplasm certain structures of definite morphological entity; they are generally referred to as cytoplasmic inclusions. The most important of these inclusions with regard to plants are the *plastids*, a common example of which is the *chloroplast* containing the green colouring matter of plants. The other important

inclusions are very minute structures which are granular or rod-shaped or threadlike in appearance. They are made visible by special methods of fixation and staining and are known as *chondriosomes* or *mitochondria*. Except that they have a semi-fluid consistency their chemical and physiological nature are at present uncertain.

The nucleus.—The nucleus is generally assumed to be the controlling centre of the entire cell and is believed to be situated in the most active region of the cell; in a growing root hair it is usually found to be occupying a position near the tip.

The chief structural parts of the nucleus are the nuclear membrane, the nuclear sap and the chromosomes. These three constituents are always present. In addition bodies known as nucleoli are also often present. The nuclear membrane has been shown by microdissection to be a definite structure with physical properties. The shape of the nucleus depends upon the properties of the nuclear membrane. Most nuclei are approximately spherical but some may be ovoid also. The *nuclear sap* is usually a clear fluid; its viscosity has been determined in one case to be about twice that of water and this is probably typical of most nuclei. In some, however, the nuclear sap may be a solid gel. The *chromosomes* are usually indistinguishable in living nuclei during the resting stage. They become visible during division. The chromosomes are thread like. They are characteristically chromatic. They are the bearers of hereditary characters. Nearly all the resting nuclei contain one or more true *nucleoli*. In the liv-

ing nucleus the nucleolus appears as a dull, viscous droplet, usually round but frequently irregular in shape. It commonly shows an affinity for dyes. Its chromaticity, however, undergoes marked alterations during the course of nuclear division.

Chemical and Physical Properties of the Protoplasm

The first cells seen were in 1665 by Robert Hooke but these were dead cells. It was much later that the importance of protoplasm was realised. The matter that constitutes "Life" is known as *protoplasm;* and, therefore, the study of the chemical and physical structure of this substance is of paramount importance; for without the knowledge of the properties of the protoplasm, the causes of growth, reproduction, senescence and death will ever remain unintelligible.

Chemical Constitution.

Next to nothing is known regarding the chemical constitution of the *living* protoplasm. So far, we have only known the chemical constitution of a *dead* cell for when it is subjected to chemical analysis the cell gets killed and rapid physico-chemical changes set in.

The chemical analysis of the protoplasm of plasmodia which contains pure protoplasm reveals that it has 75% water and 25% dry matter. The dry matter consists of both inorganic and organic compounds. The inorganic compounds consist chiefly of the chlorides, phosphates, sulphates and carbonates of magne-

sium, potassium, sodium, calcium, and iron, and the organic of chiefly proteins, carbohydrates and fats.

The following is the proportion of the dry matter in the Myxomycetes:—

Nucleo proteins .	...	40 %
Other ,, ...	...	15 %
Amino acids ...	...	1·5 %
Carbohydrates and fats	...	24 %
Salts	...	7 %
Other substances ...	...	12·5 %
Total	...	100

A mixture of these is present in a complex colloidal form. On the death of the protoplasm its weight is not decreased; and thus it is supposed that no element or compound is lost but that disorganisation of the living matter only takes place on death.

PHYSICAL PROPERTIES.

Seifriz rightly says that any protoplasm essentially consists of three types of systems viz.,

(*a*) True solution of salts, carbohydrates, amino-acids and other water soluble substances,

(*b*) Emulsion of lipides, and

(*c*) Dispersion of organic substances, mostly proteins, which form jellies.

It is this last system which is by far the most important for it particularly characterises the living tissues. Items under the group (*b*) and (*c*) come within the category of colloidal systems and thus a knowledge of the colloidal state of matter becomes exceedingly important to the study of the living protoplasm. Matter is said

to be in a colloidal state when it is permanently dispersed, and the individual particles though larger than molecules cannot generally be seen under the high power of a microscope. Thus the particle size is the characteristic of the colloidal state. The size, however, varies and ranges between 0.1 μ to 1 mμ as explained in the chapter dealing with the colloids. This is then the world of colloidal dimensions, which in a picturesque language Findlay describes as the " twilight zone of matter."

The physical characteristics of the protoplasm show that it is a viscous slimy substance possessing considerable powers of cohesion. The present known facts also indicate that the protoplasm is a colloidal solution of the emulsion type, with a polyphase colloidal system. It will, therefore, be not out of place to deal briefly with the chemistry of colloids.

However, with our present day knowledge it will be impossible to interpret the protoplasmic organisation in purely physical terms for it is too intricate: being the basis of life itself.

The Colloidal State

The word colloid is derived from the Greek word " kolla " meaning glue which is an example of this class of matter.

The beginning of colloidal chemistry really dates from 1830 when Graham first published a paper on " The effects of animal charcoal on solutions." Thus Graham has been rightly called the father of colloid

chemistry. Graham divided chemical compounds into *crystalloids* and *colloids*: the former pass through parchment paper while the latter do not. The same matter can exist in either a crystalloidal or colloidal state depending upon its condition. Thus it is the state of matter and not the kind of matter.

In a general way one could say that the substances in true solution exist in molecular or ionized form, while they are in aggregates of molecules in a colloidal state. This is not wholly true, as we find in the case of egg albumin, which in solution is in colloidal form though it is in molecularly dispersed state such as one finds in a true solution. This is so because of the large size of the molecules.

Ostwald has defined the boundaries as follows:—

Matter in mass	Colloids	Molecules and ions
	·1μ	1·0mμ

One could, therefore, say that it is the size which determines the difference between the colloid and the crystalloid.

(*a*) The Dispersal Phenomenon of a Colloid.

When one substance is distributed through a mass of a different substance, the mixture is a two phase system consisting of (*a*) the *dispersed phase* and (*b*) the *dispersion medium* or the *continuous phase*.

Thus we may have the following:—

Internal or dispersed phase	External or continuous phase	Example
1. Gas	Liquid	Foam
2. Liquid	Gas	Fog
3. Liquid	Another immiscible liquid.	Milk
4. Liquid	Solid	Jelly
5. Solid	Gas	Tobacco smoke
6. Solid	Liquid	Gold sol
7. Solid	Solid	Ruby glass

In certain cases, the internal phase is not absolutely insoluble in the external phase. In such cases the dispersed phase can be a solid containing a certain amount of the solvent and the external phase a dilute solution of the solid. These are called *lyophilic* colloids and starch solution is a case in point. In the *lyophobic* system the particles of the dispersed phase do not dissolve to any appreciable extent in the dispersion medium, as we find in gold sol.

The lyophilic and lyophobic systems are further subdivided into sols and gels as follows:

Lyophilic or hydrophilic — Sol — Gel

Lyophobic or hydrophobic — Sol — Gel

In both the cases sols are of high degree of fluidity and appear like solutions while the gels are more or less rigid.

(*b*) Some Fundamental Properties of Colloidal System.

(*i*) *Brownian movement.*—Robert Brown, a botanist, while examining pollen grains in a drop of water under the microscope, observed that they were in con-

tinuous zig-zag motion. This was inexplicable for the pollen grains had neither cilia nor had they any other means of locomotion. After about a hundred years of this discovery, Perrin, a Frenchman, was able to prove that this motion was due to the bombardment of the particles by the molecules of the liquid in which they were suspended. And as these particles were extremely small, they got one sided bombardment and hence were pushed from place to place. A large particle will necessarily get bombarded by the molecules of the liquid from all sides at once and there will be no movement. Thus the extremely fine subdivision of the particles of the dispersed phase is of paramount importance for this movement.

(*ii*) *Surface phenomena.*—These fine divisions also expose enormous surface as will be clear by the following example. Take a cube whose sides measure 1 cm. each. The total surface area of this cube will be 6 sq. cms. If this cube, now, is broken up so that each side measures 0.1 cm., then the total number of cubes in the larger cube will be 1,000 and the total surface area will be 60 sq. cms. Proceeding further if these are again subdivided to 0·01 cm. then the total surface area will become 600 sq. cms. This shows how by smaller and smaller subdivisions the surface area increases. This large surface is of vital importance in biology, as most of the reactions take place on the surface of the particles inside the protoplasm. For instance, the chloroplast, which is the seat where the food of the plant is manufactured, has a definite size

and weight. If the chloroplast is divided into particles of smaller sizes though the weight is kept the same, the total surface area is increased. Now there are certain things in solution like some organic catalysts that have a tendency to stick to the surfaces of these chloroplast particles. This sticking to the surface of a dispersed phase particle of any substance in solution is the phenomenon of *adsorption*. The greater the surface the larger amount of these organic catalysts will get adsorbed and thus the rate of the reaction will be greater.

(*iii*) *Non-diffusibility through parchment.*—If one takes a parchment membrane tied to an end of a tube which is dipping in water and a mixture of colloids and crystalloids is put inside the tube, then the crystalloids will pass out and not the colloids. This is simply because through the pores of the parchment paper the invisible molecules of the substance in solution will pass out but the particles of the dispersed phase of the colloid being larger in size will be unable to pass through the small pores of the parchment membrane.

(*iv*) *Tyndall phenomena.*—When a colloidal sol is viewed against light the solution appears clear like any other true solution. But when a beam of light is passed from one side through a colloidal sol kept in a glass tube with parallel sides, then the path of the ray becomes luminous, which is not so in the case of true solution. This is called *Tyndall phenomenon*. This is due to the polarisation of light by the particles of the dispersed phase. These particles thus become visible

and appear as bright scintillating points when seen under an ultramicroscope.

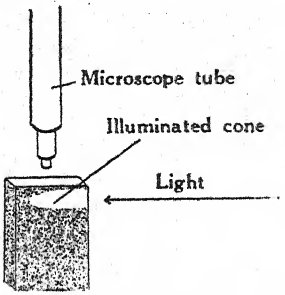


Fig. 2.—Diagram of a simple ultramicroscope. If light is passed through a collodial sol at right angles to the microscope tube, the path of the ray of light gets illuminated and light from the surfaces of the particles reach the eye through the microscope, the particles thus appear to be visible.

A simple ultra-microscope can be prepared in the laboratory by using an ordinary microscope, but instead of reflecting the light from below, a small beam of light has to be passed through one side of the glass chamber kept on the stage, as shown in the diagram (Fig. 2).

Practical Experiments

1. *Formation of a suspension.*—Precipitate a solution of barium chloride with a little sulphuric acid, shake up well the fine precipitate of barium sulphate and then leave it undisturbed for some time. Note the gradual settling of the precipitate.

2. *Formation of an emulsion.*—Shake up a little olive oil with water. An emulsion of oil in water is formed. Note that the two liquids separate in layers if the emulsion is kept undisturbed.

3. *Preparation of suspensoid solution.*—Take a few c.c. of 33% solution of ferric chloride and pour into a quantity of boiling distilled water. A colloidal

ferric hydroxide solution is formed and the colour changes to a deep brown-red. The yellow solution of ferric chloride is decomposed by excess of water with the production of a soluble colloidal form of ferric hydroxide and hydrochloric acid is set free.

4. *Preparation of emulsoid solution.*—(*a*) Take a drop of olive oil in a test tube and half fill the tube with alcohol. Shake well and pour into another test tube containing water. A fine emulsion of oil in water will be formed from which the oil will not separate.

(*b*) Make a paste of starch in cold distilled water and pour this paste in boiling distilled water and boil for a few minutes longer. A colloidal solution of starch is obtained which is faintly opalescent. (Here a concentrated solution of starch is the dispersed phase while a weak solution of starch is the continuous phase. Thus though starch is a solid its colloidal solution comes under emulsoids.)

5. *Dialysis of starch and salt solution.*—A mixture of starch and sodium chloride solutions is poured in a dialyser which is fitted, as shown in the apparatus, over distilled water. Test the liquid in the beaker after some time with silver nitrate and iodine solutions. Note the results.

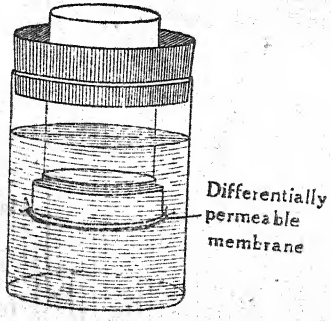


Fig. 3.—Diagram of a dialyser.

6. *Formation of reversible gel of agar-agar.*—Take a little agar-agar and pour it into a test tube containing water and boil. The agar gives a thick opalescent solution which sets to a gel on cooling. On warming, the gel again becomes a sol, and on cooling again sets to a gel. Thus the change is a reversible one.

7. *Formation of irreversible gel by using egg albumen.*—Egg albumen is liquid at ordinary temperature but on heating it becomes solid. On cooling it cannot be liquified again. Hence it is an irreversible gel.

8. *Tyndall phenomenon.*—A ray of light is passed through a colloidal solution and examined (See fig. 2).

9. *Brownian movement.*—Mount a drop ~~of very dilute India ink~~ latex of calotropis and see it under the microscope. Very small black particles will be seen moving showing Brownian movement.

10. *Streaming of protoplasm.*—Mount a leaf of *Hydrilla* and examine under the microscope. The protoplasm is seen showing streaming movements. Contrast this movement with the Brownian movement.

CHAPTER II

THE CONSTRUCTION OF PLANTS' FOOD

The Chemical Organisation of Cell Protoplasm

Before considering the process of carbon assimilation it will be necessary to discuss the foods of plants in order to understand the significance of this process in their life functions. The three important foods of plants are proteins, carbohydrates and fats. These are present in the tiny cells of plants and the metabolic flux of each is kept separate and distinct. In considering the physiology of a plant cell two things come out prominently, *viz.*,

1. How do the great variety of chemical processes which are difficult to perform in a chemical laboratory, go on in a cell? and

2. How does the protoplasm carry on all the different processes in a small cell?

The Enzymes

It is now well established that all the chemical changes that take place within the plant cell are brought about by the agency of the enzymes. An *enzyme* may be defined according to Bayliss as "a catalyst produced by living organisms." These enzymes are responsible for bringing about the various anabolic and catabolic changes of a living cell.

For the conversions of starch to sugar or sugar to starch suitable catalysts are needed. Outside the plant very high temperature and strong mineral acids are

needed for hydrolysis. But within the plant this goes on at room temperatures. This is because of the presence of the organic catalysts called enzymes. These are complex substances and their exact chemical constitution is yet unknown. The name enzyme comes from the Greek words *en zymos* which means "in yeast." It was Buchner in 1897 who first showed that if yeast cells are crushed and filtered, the filtrate can bring about fermentation, as easily as yeast cells themselves. The filtrate contains the nonliving enzyme which brings about fermentation. It has since been shown that all metabolic changes within a living cell are brought about through the agency of the enzymes. These are formed inside the cells due to the activity of the protoplasm, and in dry condition they can remain potentially active for a long time.

The enzymes are specific in their action, which means that a particular enzyme will act only on a particular substrate. Accordingly, keeping in view, the particular aspect of metabolism which they affect, the enzymes are classified as follows:—

1. *HYDROLASES*

(*a*) *Carbohydrases*—in which are included Cellulases, Diastases, Dextrinases, Invertases, Maltases, etc.

Under the group Carbohydrases come all the categories of enzymes that are associated with the carbohydrate metabolism. Under this head are then classified separate enzymes that attack each specific substrate, for instance those that attack starch and convert it to glucose

are called Diastases, those that invert cane sugar into glucose and levulose are the Invertases etc.

(*b*) *Proteases*—under this we have the three types of enzymes, Pepsins, Trypsins and Erepsins.

The Proteases take part in the protein metabolism. Pepsin occurs in the gastric juices of animals and is rarely found in plants. It hydrolyses proteins to form proteoses or peptones. The enzyme Trypsin is widely found in plants and specially in the papayas (*Carica papaya*) as "Papain." It hydrolyses proteins not only upto the peptones but splits these further to the amino acids. Erepsin has been found in the embryo of wheat, in green peas and is generally widely distributed. It digests peptones and other polypeptides to amino acids.

(*c*) *Lipases*—the enzymes of this group hydrolyse all the simple esters of the type trihydroxy alcohols and fatty acids.

The above are called Hydrolases for the metabolic reactions of the substrate within this great group comprises either the intake or removal of water. The enzymes under this group not only bring about hydrolysis, but also synthesis by condensation of carbohydrates, proteins and fats from their simpler constituents. This depends upon various factors such as the concentration of the substrate, salts, temperature, etc. Thus the very enzyme at one period may form polysaccharides and at another period may hydrolyse starches to sugars.

2. *OXIDASES*

(*a*) *Oxygenases*—These cause formation of organic peroxides.

(*b*) *Peroxidases*—The peroxidases are capable of rendering active the oxygen of peroxides. The oxygen is freed from peroxides in atomic or active form and is thus capable of causing the oxidation of certain compounds.

(*c*) *Catalases*—These enzymes are capable of decomposing hydrogen peroxide into water and molecular oxygen but do not activate the oxygen. Thus

$$2\,H_2O_2 + \text{Catalase} \longrightarrow 2\,H_2O + O_2$$

The enzymes under the Oxidases act as oxidising agents and are responsible for the various types of biological oxidations such as respiration and fermentation.

Most enzymes are very sensitive to heat and are thus called thermolabile. They are destroyed at a temperature of 100°C or even much below. Enzymes possess colloidal properties, *e.g.*, want of diffusibility through parchment. Most enzymes are soluble in water but insoluble in alcohol. This helps to bring about the separation of the enzyme which is mixed up with other chemical substances within the plant cell.

The Plants' Food

THE PROTEINS.

As during the metabolic flux the production and break down of the proteins, carbohydrates and fats, continuously go on, a knowledge of their chemical behaviour is essential.

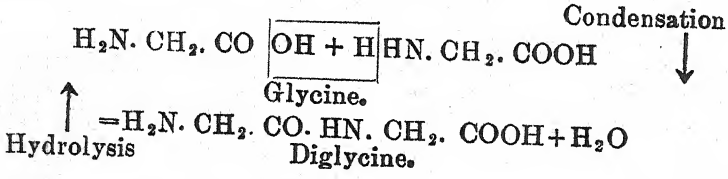
Proteins are divided into four classes:—

1. Amino-acids
2. Derived proteins
3. Simple proteins.
4. Conjugated proteins

1. *Amino-acids.*—Amino-acids are compounds which contain at least one carboxyl (-COOH) group and one or more amino ($-NH_2$) groups. Glycine $CH_2.COOH$ is one of the simplest amino acids.
NH_2

It was E. Fisher (1901) who first gave a clue to the chemical nature of the higher proteins. He showed that the higher ones were formed by the joining together of the lower proteins. Therefore one may say that the lower ones are the building stones.

Taking glycine as the simplest of amino-acids let us see how the condensation and hydrolysis are brought about.



Here OH from the acid group of one molecule of glycine and H from the amino group of the other are removed to form diglycine and water. The diglycine is also called dipeptide. The joining of the two molecules of glycine to form a dipeptide is a process of condensation, while in hydrolysis a molecule of water is

taken up and diglycine gets hydrolysed to two molecules of glycine. By the addition of more molecules tri-, tetra-, etc. glycines can be formed. Cystine, $COOH.\ CH(NH_2).\ CH_2.\ S.S.\ CH_2.\ CH.\ NH_2.\ COOH$, is another amino-acid and contains sulphur.

2. *Derived proteins.*—These include the proteins that are formed as a result of partial hydrolysis or decomposition of the simple proteins. The proteoses and peptones are examples.

3. *Simple proteins.*—These are proteins which yield only amino-acids on hydrolysis, *e.g.* albumins, globulins etc.

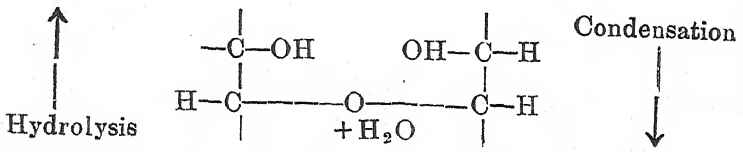
The higher proteins are colloidal in nature while amino-acids are crystalloidal. Thus amino-acids are more active. If all the four classes of proteins are enclosed in a parchment paper which is dipped in a basin of water class one represented by the amino-acids will readily diffuse out.

4. *Conjugated proteins.*—These are compounds of proteins with some other non-protein group, e.g., nucleoproteins, hemoglobins etc. In the nuclei of plant cells, true nucleoproteins do not occur but they are present as salts or esters of protein and nucleic acid.

The Carbohydrates.

These are the most important constituents after proteins. They contain carbon, hydrogen and oxygen, the last two in the same proportion as in water. *Oses* are the common name for sugars, *e.g.*, *monoses*

are the monosaccharides containing six carbon atoms, viz. $C_6H_{12}O_6$ *Dioses* or the disaccharides are the condensation products of the monoses with double the number of carbon atoms, e.g., $C_{12}H_{22}O_{11}$ Although there is much difference between proteins and carbohydrates, their hydrolysis and condensation are very similar.

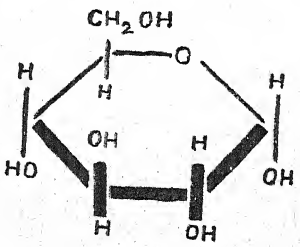


The structural formula of *glucose* is

CHO. CHOH. CHOH. CHOH. CHOH. CH_2OH.

This shows that it is an open chain compound with an aldhyde group—C—H. This is, therefore, also called

aldohexose. This sugar having four asymetric carbon atoms (CHOH), will have sixteen sterioisomeric forms according to the formula 2^4. In accordance with the latest view glucose is a closed chain compound with the following structural formula.



Similarly the fruit sugar or *fructose,* the ketohexose, is a ketone sugar, having the following structural formula. $CH_2OH.CO.CHOH.CHOH.CHOH.CH_2OH$. This has only three asymmetric carbon atoms and, therefore, according to the formula 2^3, it should have eight stereoisomeric forms. Further eight forms of the ketone sugars will also be possible if the O in the CO is on the left instead of the right. Thus, as in the case of aldohexoses, here too there should be sixteen stereoisometric forms. In all there will be thirty-two isomeric forms of hexoses. Recent researches have, however, shown that due to the formation of ethylene and butylene oxides, the number of forms can be greatly increased.

Under the monosaccharides are hexoses and pentoses. Pentoses have five carbon atoms, having the following structural formula $CHO.CHOH.CHOH.CHOH.CH_2OH$. This is also commonly found in plants. It will have eight isomers; while its ketone counter part $CH_2OH.CO.CHOH.CHOH.CH_2OH$, will have also eight.

Sucrose or cane sugar $C_{12}H_{22}O_{11}$ is a condensation product of a molecule of glucose with a molecule of fructose, with the consequent elimination of water. It is commonly found in most plants. The ordinary sugar that we eat is cane sugar and is extracted from the cane sugar plant, from whence it derives its name.

Starch.—$(C_6H_{10}O_5)n$. This is a polysaccharide and is supposed to be formed by condensation of a

number of hexose sugars. It is colloidal in nature, and thus it is osmotically inactive. It forms the main reserve of food stuffs in the plant kingdom, and is found in practically every region of the plant, *e.g.*, leaves, petioles, stems, roots, seeds etc. There are two polysaccharides in starch grains, *viz., amylose* which is soluble in water and is stained blue with iodine, and *amylopectin* which is insoluble in water and is stained violet with iodine. The latter forms the outer layer. The swollen amylopectin in solution gives the pasty condition when a little water is added to starch powder.

In plants, there is a continuous flux of up grade and down grade sugars, brought about by the agency of plant enzymes: the type of flux, *e.g.*, up grade or down grade depending upon the condition of the cell at the moment. The following table shows the probable course of the up grade and down grade flux.

	Enzyme	*Cellulase*	*Diastase*	*Galactase*	*Glycogenase*	
	Polysaccharide $(C_6H_{10}O_5)n$	Cellulose	Starch Dextrin	Galactan	Glycogen	
Down grade flux	Disaccharide $C_{12}H_{22}O_{11}$		*Maltase* Maltose	*Lactase* Lactose		Up grade flux
	Monosaccharide $C_6H_{12}O_6$	Glucose + Mannose	Glucose	Glucose + Galactose	Glucose	

It was Croft-Hill who first showed that even outside the plant, the monosaccharides can get condensed into the higher forms of carbohydrates, thus showing that the same enzyme can work both ways: its particular action depending upon the condition of the substrate.

In one of his experiments Croft-Hill used the enzyme maltase with a solution of 40% glucose. In due course he got the disaccharide maltose in solution. But it took him seventy days to get to the equilibrium point.

$$\underset{85\%}{2C_6H_{12}O_6} \longrightarrow \underset{15\%}{C_{12}H_{22}O_{11}} + H_2O.$$

The equilibrium was reached when in his solution there was 85% of glucose and 15% of maltose.

Similarly, using the enzyme invertase with a solution of glucose and fructose, cane sugar can be formed.

Cellulose.— $(C_6H_{10}O_5)n$. This is a polysaccharide and is the primary constituent of the cell wall. Like starch, on hydrolysis, it yields glucose and mannose, and therefore may be regarded as a condensation product of these substances. Its formation is brought about by the probable action of the enzyme cellulase or cellobiase.

In a young meristematic cell when the cell-wall is just being formed cell-wall material of cellulose is laid in separate bits inside the protoplasm. Later on, they are joined together and thickenings take place. As the cell-wall is laid within the protoplasm and not on it a close-parallel may be found to the formation of starch.

It is very rare that the cellulose wall is hydrolysed in the plant kingdom. But there are exceptions. Cacti have been seen to thin down if kept for a long time in darkness. Here the cellulose gets hydrolysed.

Hemicelluloses.—Apart from celluloses, there are other substances, found as reserve in the cell-walls of certain plants, *e.g.*, the seeds of *Lupinus luteus, Tropaeolum*, date etc. In the date the hemicelluloses, are synthesised from pentoses and hexoses such as mannose, glucose and galactose.

Gums and *mucilage* are met with in some plants; they have a similar composition to that of hemicelluloses, but involve pentose sugars in their composition.

Fats and Oils.

In every plant cell along with carbohydrates and proteins, fats or substances allied to fats are present. They also form an essential structure of the plant protoplasm. They occur abundantly in spores and seeds as reserve food material. In many fungi oils are found in spores and mycelia. In some higher plants, seeds have a very high percentage of oils such as the seeds of olive oil, nuts and almonds.

The true fats are glyceryl esters of the fatty acids of which the glycerides of caproic acid $C_3H_5(OOC C_5H_{11})_3$ and of oleic acid $C_3H_5(OOC.C_{17}H_{33})_3$ are most commonly found. On hydrolysis fats yield glycerol and the corresponding fatty acids, *e.g.*:—

$$\begin{array}{l} CH_2.OOC.C_{17}H_{33} \\ | \\ CH\ OOC.C_{17}H_{33} \\ | \\ CH_2.OOC.C_{17}H_{33} \\ \text{Triolein} \end{array} + 3H_2O = \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \\ \text{Glycerol} \end{array} + \begin{array}{l} HOOC.C_{17}H_{33} \\ | \\ HOOC.C_{17}H_{33} \\ | \\ HOOC.C_{17}H_{33} \\ \text{Oleic acid} \end{array}$$

In addition to these true fats we often get waxes. Here the place of glycerol is taken by a monohydric alcohol.

The enzyme lipase effects the hydrolysis of fats. And as in the case of carbohydrates and proteins its synthesis is also effected by the same enzyme.

Oil ⟵ fatty acid + glycerine
38% 62% (at equilibrium).

Practical Experiments

TESTS FOR PROTEINS.

1. *The Xanthoproteic reaction.*—To a few c.c. of the protein solution in a test tube add about 1/3 of its volume of pure strong nitric acid. A white precipitate is formed, which on boiling turns yellow, and partly may dissolve to give a yellow solution. Cool under the tap, and add ammonia till the reaction is alkaline. The yellow colour becomes orange. The precipitate is due to the fact that metaprotein is formed.

2. *Millon's reaction.*—To a few c.c. of protein solution add about half its volume of Millon's reagent. A white precipitate is formed. On warming, the precipitate turns brick-red, or disappears and gives a red solution. The white precipitate is due to the reaction of mercuric nitrate on the proteins.

3. To a few c.c. of the protein solution add about 1 c.c. of 40% sodium hydroxide and one drop of 1% copper sulphate. A violet or pink colour is produced.

4. *Micro-chemical reaction.*—Cut a section of a pea-seed soaked in water. Treat the section with a 5% copper sulphate solution for 30 minutes. Wash it in water and mount in a drop of 50% potassium hydroxide

solution. The section is stained red, showing the presence of proteins.

TESTS FOR GLUCOSE.

1. *Moore's test.*—Boil a little of glucose solution with an equal volume of sodium hydroxide solution. A yellow colour is developed which is due to the formation of a condensation product of sugar.

2. *Trommer's test.*—Add a few drops of a 1% copper sulphate solution to 2 to 3 c.c. of 5% caustic-soda solution. A blue precipitate of cupric hydroxide is formed. Add now 2 to 3 c.c. of glucose solution and the precipitate will dissolve. On boiling the blue colour disappears and a brick red precipitate of cuprous oxide is formed.

3. *Fehling's test.*—Boil a few c.c. of freshly made Fehling' s solution in a test tube and note that it is unaltered. Then add an equal quantity of the glucose solution and boil again. A brick red precipitate of cuprous oxide is formed.

TESTS FOR CANE SUGAR.

1. *Moore's test.*—A negative result is obtained.

2. *Fehling test.*—No reduction takes place.

3. *Hydrolysis.*—To a few c.c. of the solution add a drop of strong sulphuric acid and boil for two minutes. Then neutralize with caustic soda using litmus as indicator. Boil again and add Fehling's solution drop by drop. A reduction takes place owing to the inversion of the cane sugar by sulphuric acid.

TESTS FOR STARCH.

1. Add a few drops of iodine solution to a little of starch solution. A blue colour is obtained. Heat the solution, the blue colour disappears, but reappears on cooling.

2. Add an equal volume of alcohol; the starch is precipitated.

3. *Microchemical test.*—To a section of soaked pea-seed add iodine solution. It stains dark blue, showing the presence of starch grains.

4. Examine and sketch the various kinds of starches found in the following:—

(*a*) *Potato.*—Starch grains large. Show clear lamination; excentric, hilum clearly visible.

(*b*) *Bean.*—Starch grains oval or circular, slightly flattened, medium size, lamination clear, and uniform, hilum centric and the structure concentric. Radial clefts pass out from hilum.

(*c*) *Wheat.*—Lamination not clear, grains circular, flattened, hilum central.

(*d*) *Oat.*—Compound grains, individual component grains polygonal. Lamination not visible.

(*e*) *Euphorbia.*—Grains dumb-bell shaped.

STUDY OF ENZYME ACTION.

1. Treat a little starch solution with your saliva. Shake it for some time and then boil with equal volume of sodium hydroxide. A yellow colour is developed on account of the conversion of starch into sugar by the enzyme ptyalin in the saliva.

2. Prepare a solution of the enzyme diastase. Put a few drops into a weak solution of starch. Keep it warm by rubbing the test tubes with your hands. Test it for starch. Note that no blue colour develops with iodine. Test it with Fehlings. It gives a test for sugar.

Enzymes are destroyed by heat. So they will have no action in experiments 1 and 2 if they are used after heating.

TESTS FOR FATS AND OILS.

1. Try the solubilities of the oil in water and petrol ether. It is soluble in petrol ether and insoluble in water.

2. Add Sudan III to a drop of oil. The oil globules turn red.

3. Cut a T. S. of *Citrus* leaf and examine. There are small globules of essential oil lying here and there in the cells. Though these are not fats they turn red with Sudan III.

4. Cut a T.S. of castor oil seed. Stain with Sudan III and examine. Oil globules in the cells turn red.

CHAPTER III

THE METABOLIC BIOGRAPHY OF A PEA

The metabolic stages of a pea or any other plant may be divided into five phases, *viz.*

1. Dry seed
2. Germination
3. Seedling
4. Vegetation
5. Reproduction

Dry seed.—The examination of the cotyledons in a pea (*Pisum sativum*) shows that protein is present in every cell of the embryo. It is an exalbuminous seed and three per cent. of its dry weight is protein. If a section of a seed is taken and treated for thirty minutes with a five per cent. copper sulphate solution and then washed with water and mounted in fifty per cent. potassium hydroxide solution, the section will be stained pink or violet. This indicates the presence of proteins. If to another section of a soaked seed iodine in potassium iodide solution is added it is stained dark blue showing the presence of starch grains. The apices of plumule and the radicle contain only a colloidal mass of protoplasm.

Germination.—On soaking the seed in water it swells and by the extension of the middle of the radicle causes the primary root to come out which penetrates further into the soil. Soon the elongation of the epicotyl *i.e.,* the region between the cotyledons and the

first leaves takes place and the plumule is pushed up through the soil into the air. Thus the seedling stage is reached.

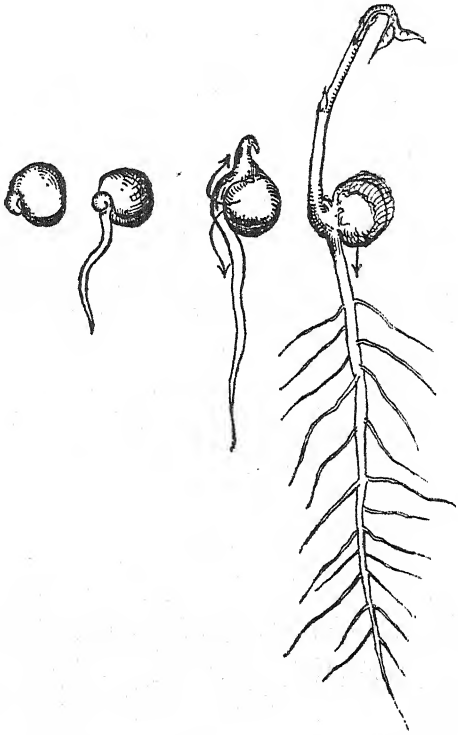


Fig. 4.—Stages in germination of pea seedings. The arrows indicate the directions of translocation of sugars and amino acids.

Seedling.—When germination starts the proteins and starches which are present in the seed as stored food material, get broken down by enzyme action, to amino-acids and hexose sugars respectively. They are then translocated towards the growing plumule on the one hand and the radicle on the other. Arrows in figure 4

indicate the direction of the passage of sugars and amino acids towards the growing radicle and plumule. Some of these sugars and amino acids remain in cells just behind the growing tip of the radicle in the vacuoles causing osmosis and expansion of the cells. Yet a portion travels further to the meristematic region, where a part gets oxidised to give the necessary energy to the growing cell and the rest gets converted by the energy of the cell into its living structure, the protoplasm. What is true for the radicle is true for the plumule also. When the seedling emerges out of the ground and the first assimilating leaves appear the drain of sugars and amino-acids from the seed stops.

The young green leaves now start to form their own starches and proteins and these now get hydrolysed to sugars and amino-acids to be translocated to meristematic regions.

Vegetation.—Then follows the stage of free vegetative activity and the plant grows in all its luxuriance.

Reproduction.—After a period of vegetative activity the plant flowers and the seed is formed. In the seed the sugars, amino-acids and glycerides are now poured in from either the manufacturing centres like the leaves or the storage organs of the plants such as the tubers or the medullary rays etc. These are then condensed into starches, proteins, and fats, and stored in the cotyledons as such to be again used at the time of the germination of the seed.

CHAPTER IV

PROBLEMS OF NUTRITION

Distinction between Autotrophic and Saprophytic Modes of Nutrition

In the *autotrophic* mode of nutrition, plants manufacture organic substances from inorganic matter, while *saprophytic* plants utilize only organic matter. They are more or less like animals who cannot manufacture their own food from raw materials. To say that plants live on inorganic matter and animals on organic, is not exactly true, for both live on organic matter. The former, however, manufacture it and then live on this manufactured product while the latter, unable to manufacture it, simply live on others.

The uses of food are twofold, *viz.*:—

1. It provides materials for the construction of the body, and

2. It provides energy for carrying on the vital functions.

The animals and saprophytic plants, which are devoid of chloroplasts, live on autotrophic plants. So we may say " All flesh is grass."

PHOTOSYNTHESIS

Brief Historical Resume

It was Priestley in 1772 who was the first to show that the vital air meaning oxygen was vitiated by animals.

Ingen-Housz, who was a physician to the Emperor of Austria, got interested in Priestley's papers and showed in 1779 that light was an essential factor for the purification of air by plants.

Dutrochet (1837) further showed, that the green part was essential for photosynthesis, while Sachs in 1887 showed that the first visible product of photosynthesis was starch. Sachs further showed that the rate of photosynthesis increased, up to a certain point, with the increase of temperature and thereafter decreased. According to him the temperature at which photosynthesis just started was *minimum,* where it attained its highest form it was *optimum,* and where again it just was able to continue it was *maximum.* At this period, however, the idea of photosynthesis was not very clear until the brilliant researches of F. F. Blackman and his collaborators showed, that distinct factors were involved in this process.

Factors in Photosynthesis

In order that photosynthesis may proceed in a healthy plant, it is necessary that light should fall on chlorophyll bodies in the presence of carbon dioxide and water. Temperature is an important factor also. So that we may enumerate the following as some of the important factors.

External:—

1. Carbon dioxide

2. Light.
3. Temperature
4. Water.

Internal:—

1. Chlorophyll
2. Protoplasmic factor
3. Products of photosynthesis

Various Phases in Photosynthesis.—Before going into the details of the various factors an understanding of the various phases involved in photosynthesis is essential.

The first phase is the gas exchange between green tissues and the surrounding air, whereby carbon dioxide gets absorbed by the cell sap. This is followed by the absorption of radiant energy by chlorophyll bodies by which the kinetic energy is utilized in the break down of the carbon dioxide and water, with the consequent production of organic substances such as sugars, with a high potential energy, and the elimination of oxygen. This is followed by condensation of sugars. We thus see that there is a physical mechanism for gas exchange, a series of transformation of energy and compounds and the ultimate deposition of food material. The chemical equation may be written thus:—

$$6CO_2 + 6H_2O + 673 \text{ kg. cal.} \longrightarrow C_6H_{12}O_6 + 6O_2$$

We shall now deal with each factor which governs the rate of carbon assimilation in the great plant factory.

CARBON DIOXIDE.

The plant draws this raw material from two sources, viz.:—

1. The products of respiration, and
2. The atmosphere.

Taking the first case it is an established fact that every living cell respires and in the normal type of respiration carbon dioxide is given out. This carbon dioxide can be reconverted into carbohydrates with the elimination of oxygen in the presence of light. But normally the process of assimilation is far more rapid than respiration, thus necessitating intake of carbon dioxide from the atmosphere.

The atmosphere contains about ·03 per cent. carbon dioxide only. This amount is almost infinitesimal when we contemplate the results of its use. Naturally this limited amount suggests the necessity of broad leaf surfaces and proper distribution of chlorophyll. An ordinary plant within wide limits uses up 13 c.c. of carbon dioxide per hour per decimeter leaf surface. This amount is present in a column of air ten feet high with an area of the leaf at the base. This amount is astonishingly large, considering that most of the leaf area is covered up with epidermis and cuticle. It has been shown by exposing a leaf of the same area as a solution of potassium hydroxide that the rate of carbon dioxide absorption is the same.

This may be explained by assuming that leaf is a multiperforate septum. The aggregate area of the stomata, which only can allow carbon dioxide of small

concentrations as is present in the atmosphere to enter, is about 1 per cent. of the area of the leaf. But the flow of carbon dioxide into the interior of the leaf is not straight; the lines of flow take a curve.

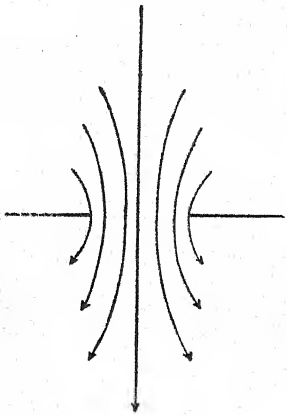


Fig. 5.—Diagram to illustrate the lines of flow of a gas through a small aperture in a septum.

The above diagram shows that though it is a multiperforate septum yet carbon dioxide flows in as if there was no hindrance. This is only possible if the maximum distance of each perforation or stoma is ten times the diameter of the perforation. The mechanism of the opening and closing of the stomata will be dealt with in the chapter dealing with the ascent of sap.

The amount of carbon dioxide present in the atmosphere is sufficient for the needs of the plant throughout all times, though it has been found that if air contains 1 to 10 per cent. more carbon dioxide, it would be beneficial. In Germany the carbon dioxide of furnaces in the industrial areas is carried by means

of underground pipes to agricultural lands outside the cities where it serves as aerial manure.

The amount of carbon dioxide in the atmosphere is pretty nearly constant for the forces governing the supply and demand are somewhat regulatory, though according to geological evidence it is not absolutely so. Man alone gives out practically 50,000,000 tons of carbon dioxide daily and the burning of coal, wood, oil etc. returns to the air several billion tons of carbon dioxide every year. With the rapid circulation of the air the carbon dioxide is evenly distributed throughout, and plants all over the globe get equal facility.

F. F. Blackman (1905) has shown that if all the factors governing the rate of photosynthesis are in excess except the factor of carbon dioxide, then the rate of photosynthesis will depend upon the concentration of this factor alone. Thus with the increase of carbon dioxide in the atmosphere the rate of photosynthesis will rise till very high concentrations are reached causing narcotic effect upon the cell protoplasm.

LIGHT.

(*a*) *Intensity of Light.*—As light is the prime source of energy for photosynthesis, it is natural that with increased intensity the process also augments. This was clearly demonstrated by Blackman and Matthaei in 1905. Previous to these workers all the other factors necessary for photosynthesis were not known and thus the results were conflicting. Blackman and Matthaei in their experiments kept tempe-

rature and carbon dioxide supply in excess. They noticed that as the light intensity was increased from minimum the rate of photosynthesis also increased. This increase, of course, stopped after a certain intensity for the chlorophyll failed to absorb and hold all the light in very strong illumination. Strong light also tends to disorganise the chlorophyll apparatus. In this matter leaves may be divided up into two classes:—

1. The sun leaves
2. The shade leaves

The two differ in both anatomical and physiological characteristics. The leaves of sun plants are thicker, due to the greater development of the palisade parenchyma.

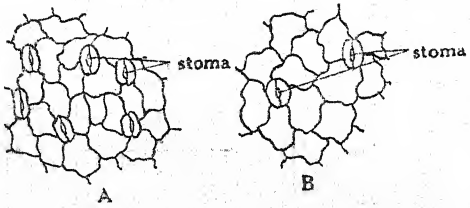


Fig. 6.—Surface view of epidermis of leaves of *Nasturtium* growing (A) in sun and (B) in shade.

The cells too are of smaller size and the number of stomata is greater. The same plant may have sun leaves and shade leaves on it according to whether the leaf grew in the sunny or the shady side, as can be seen in *Nasturtium*. Naturally with less amount of palisade parenchyma the amount of chlorophyll is also less in shade loving plants. Thus they are able to

absorb less of the solar radiations. Blackman showed that in low light intensities equal areas of sun and shade

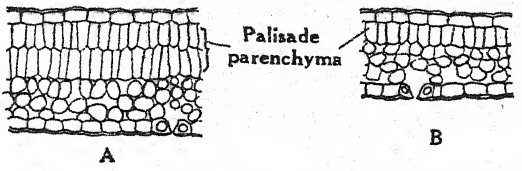


Fig. 7.—Cross sections of leaves of *Nasturtium* growing (A) in sun and (B) in shade.

leaves assimilated to the same extent. But in high light sun leaves assimilated more.

(*b*) *Different Wavelengths of Light.*—It has been found that assimilation of carbon takes place throughout the visible part of the spectrum and also slightly in the ultraviolet, *viz.*, between 330 and 760 m μ. The experimental evidence goes to show that with equal intensities of incident light photosynthesis is influenced by wavelengths, being greatest in the red end of the spectrum. The absorption spectra of different wavelengths of light by chlorophyll, however, show great absorption at red and blue-violet regions and very little at green. We thus see that the rate of photosynthesis does not quite agree with the absorption coefficient of the chlorophyll.

There is evidence that some assimilation can take place in the infra red region also. If a brass plate with a piece of ebonite inserted in it, be placed on a leaf exposed to the sun, some amount of starch formation can be noticed. Ebonite is transparent to infrared but opaque to visible light.

Temperature.

Photosynthesis being both a photo-chemical and a chemical reaction, it follows that both light and tem-

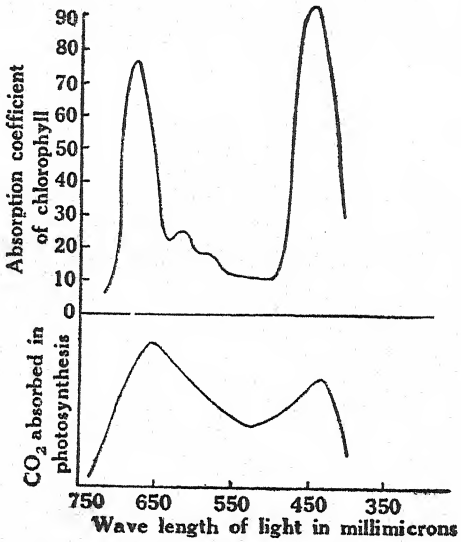


Fig. 8.—Diagram illustrating the photosynthetic activity in light of different wavelengths.

perature will profoundly affect it. According to Vant Hoff's law, the rate of a chemical reaction approximately doubles with every increase of 10 °C. This acceleration of a reaction with the 10° C. increase of temperature is also designated by Q_{10}, which is also called *temperature coefficient.* Vant Hoff's law, within narrow limits, *e.g.*, between 0° and 30 °C. is applicable to assimilation of carbon. Beyond this temperature, rapid falling off of assimilation with each successive hour takes place.

The threshold temperature of photosynthesis varies a good deal; this may be low as $-20°$ C. in the case of certain lichens and as high as $+9°$C. in the case of certain tropical plants.

WATER.

Another important external factor governing the rate of photosynthesis is water. It affects the rate of photosynthetic process both directly and indirectly.

1. In the direct action it combines chemically with the carbon dioxide of the atmosphere to form the carbohydrates.

2. Indirectly (a) it helps to maintain the turgidity of the leaf cells and (b) it enables the stomata to remain open to facilitate gaseous exchange.

It has been shown that a reduction of 53 to 78 per cent. of photosynthesis takes place when water content of the leaves of *Bidens tripartita* is reduced by 43 to 44 per cent. Dastur has also proved that there is a direct correlation between the decrease in the rate of photosynthesis and the fall in the water content per unit area of the leaf.

The decrease in the rate in photosynthesis may also take place in very strong light which helps to increase evaporation and thus closure of the stomata may take place (refer to page 139).

CHLOROPHYLL.

The vast number of synthetic products formed inside the plants, upon which animal life depends, is brought about in the great plant laboratory by the help

of its green colouring matters. The origin of these green colouring matters is still a mystery. According to Guilliermond there are minute structures in all the living plant cells called chondriosomes, which arise from pre-existing chondriosomes. During development these not only form plastids but also other metabolic products. Those plastids that have the green colouring matter are called chloroplasts. The plastids themselves are hollow, flattened, ellipsoidal structures with a central vacuole. The green pigments are evenly distributed throughout the ground substance, though there is some evidence that they occur only at the periphery. Willstätter has shown that the leaf pigments are in a colloidal state in the living plants. For the development of the green pigment in the plastid, light, adequate temperature, and a small quantity of iron are essential.

When ordinary sunlight falls upon a green leaf all the various parts of the visible spectrum are not absorbed. When the light which has passed through a chlorophyll solution is analysed into its colours by a prism, it is seen that there are certain dark bands which show the regions of the missing wavelengths. These are the radiations absorbed by the chlorophyll solution.

Figure 9 shows the absorption spectra of the various plant pigments. The principal absorption bands lie at the red end and the blue-violet end. The absorption bands at the red end are principally due to the chlorophyll pigments while the absorption at the blue-violet end is due to the carotinoid pigments.

Chlorophyll can be extracted from leaves, without changing its composition by 80 per cent. acetone solution. It forms a rich green solution with a blood red fluorescence.

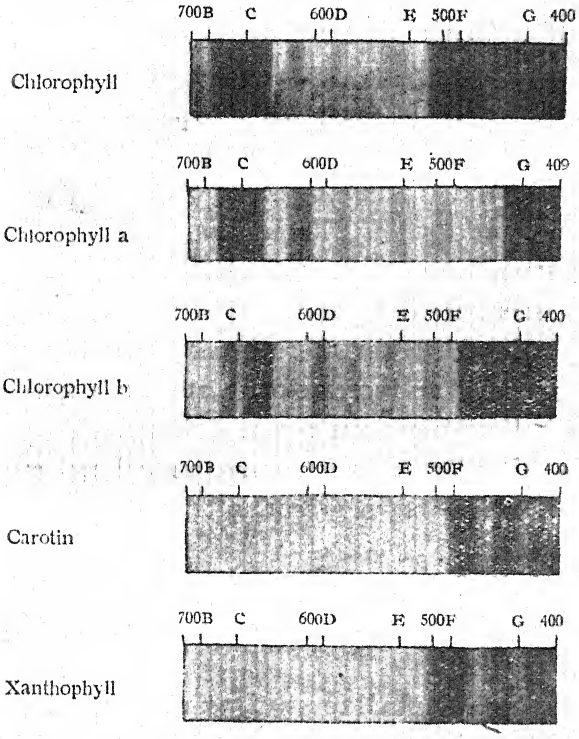


Fig. 9.—Absorption spectra of plant pigments.

Before Willstätter's work it was not clear as to how many pigments were there in the chloroplasts. It has now been shown by him that there are four pigments, *viz.*, 2 green and 2 yellow.

The two green pigments are:—

Chlorophyll *a*, $C_{55}H_{72}O_5N_4$ Mg which constitutes ·6 per cent. of the dry weight of the leaf, and

Chlorophyll *b*, $C_{55}H_{70}O_6N_4$ Mg which constitutes ·2 per cent. of the dry weight of the leaf.

Brown and red algae do not contain chlorophyll *b*. Its place seems to have been taken by two other newly discovered forms of green pigments *viz.*, chlorophyll *c* and chlorophyll *d*. Strain and Manning (1943) found that brown algae and diatoms have chlorophyll *c* and red algae have chlorophyll *d*. The composition of these are as yet unknown.

As will be seen, the difference between chlorophylls *a* and *b* is, that in the latter there is a reduction of 2 atoms of hydrogen and an increase of an atom of oxygen.

The two yellow pigments are as follows:—

Carotin, $C_{40}H_{56}$. It is orange in colour and constitutes ·03 to ·08 per cent. of the dry weight of the leaf, and

Xanthophyll $C_{40}H_{56}O_2$. It is yellow in colour and constitutes ·07 to ·1 per cent. of the dry weight of the leaf.

Carotin and xanthophyll are hydrocarbons. There is a close similarity between the chlorophyll pigments of the leaf and haemoglobin which is the red colouring matter of the blood. But whereas, in the former magnesium forms part of the structure of the molecule in the latter iron does so.

Outside the plants these pigments are easily destroyed by light and oxygen.

The chloroplast takes up the radiant energy and converts it to the right kind of energy, which in turn causes the reduction of carbon dioxide, without itself getting impaired. Thus chlorophyll is a photocatalyst.

Willstätter has shown that the rate of photosynthesis is to a large extent dependent upon the amount of chlorophyll. Shade plants with thin leaves containing a single layer of palisade parenchyma can absorb less light than a sun leaf with a double layer of palisade parenchyma. In strong light the sun leaf can assimilate carbon dioxide at a greater rate than the shade leaf.

Protoplasmic Factor of Photosynthesis.

Protoplasmic factor is another internal factor of photosynthesis. It is different from chlorophyll factor but may be enzymatic in nature. Irving (1910) found that in the case of young leaves of barley and *Vicia Faba* which were just fully green the efficiency of photosynthesis went on increasing with age but without further increase in chlorophyll. Briggs (1922) termed it as "Photosynthetic potentiality" and concluded that this unknown factor increased in a newly growing assimilating tissue with age irrespective of light or darkness.

Products of Photosynthesis.

Carbohydrates and oxygen are the chief products of photosynthesis. The latter, however, as soon as it is formed, escapes into the atmosphere and thus does not affect the rate of photosynthesis. Carbohydrates,

on the other hand, remain in the leaf cells, and consequently affect the rate of carbon assimilation.

According to the law of mass action chemical action decreases as the product of its activity increases. If the initial rate is to be maintained then the accumulated substances must be removed. In the morning at sun-rise the amount of sugars in the leaf cells is low, thus there is active photosynthesis; but towards noon, due to the accumulation of the photosynthesised sugars the rate falls.

The Interaction of Factors

We have so far dealt with the effect of individual factors on the assimilation rate of plants. It was F. F. Blackman who after a series of extensive researches established in 1905 the Law of Limiting Factors, which, with certain minor modifications, holds good even today. The law runs thus: " When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is controlled by the pace of the slowest factor." Slowest factor stands for the factor in the minimum. We have already seen that the rate of photosynthesis is governed by light, carbon dioxide supply, temperature, water, chlorophyll etc. Now suppose all these factors are present in large excess except carbon dioxide, then according to the law of limiting factors the rate of photosynthesis will be governed by the concentration of carbon dioxide alone.

A time may come, however, when by progressive increase of carbon dioxide this factor may become in

excess of the requirements of the plant and thus a further increase will no longer augment the photosynthetic rate. Here it may be that light now becomes limiting. If it is so, then according to the Law of Limiting Factors, an increase of this factor will augment further the rate of photosynthesis. If, however, photosynthesis does not increase, then obviously some other factor must be limiting by increasing which photosynthetic rate will increase.

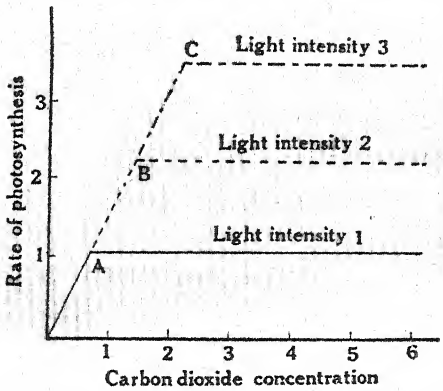


Fig. 10.—Diagram illustrating Blackman's law of Limiting Factors. Explanation in text.

Figure 10 graphically explains this phenomenon. The rate of photosynthesis increases up to a point A, with the increase of carbon dioxide concentration, when a certain light intensity 1 is administered. With light intensity 1 photosynthesis does not increase beyond the point A, even though carbon dioxide concentration may be increased. Here, then, at A and beyond carbon dioxide concentration has become in excess for the light

intensity of 1. If, however, light intensity is doubled to 2 then the rate of photosynthesis will again go up with the increase of carbon dioxide concentration as shown by the rise from A to B. Thus we see that with light 1 carbon dioxide concentration beyond A is relatively in excess but it becomes limiting when double light is given.

At B again light becomes limiting. Thus any increase of carbon dioxide cannot augment the rate of photosynthesis. When light is increased threefold the rate of photosynthesis is again increased.

At the points A, B and C in figure 10, according to Blackman there is a sharp inflection point: so that the rate of increase is linear with increased concentration of CO_2 and at the limiting light intensity the increase is suddenly arrested.

Blackman's law of limiting factor during recent years has been modified. Boysen-Jensen (1918), working on *Sinapsis alba* found that the increase in the rate of photosynthesis, when a limiting factor is increased, does not show a rise in linear proportion but, that, with increasing concentration of a particular limiting factor the rate of increase of photosynthesis diminishes. Thus instead of getting a curve of a type of Blackman's (Fig. 10) a smoothed curve is obtained.

With the help of more sensitive apparatus Warburg (1919) found a similar type of curve using the unicellular alga *Chlorella*. Harder (1921) (Fig. 11) working on *Fontinalis* also found that when only one factor is changed the curve representing the rate of photosynthesis

is of a logarithmic type. Harder further found that by varying either light intensity or concentration of CO_2 the rate of photosynthesis could be altered and he there-

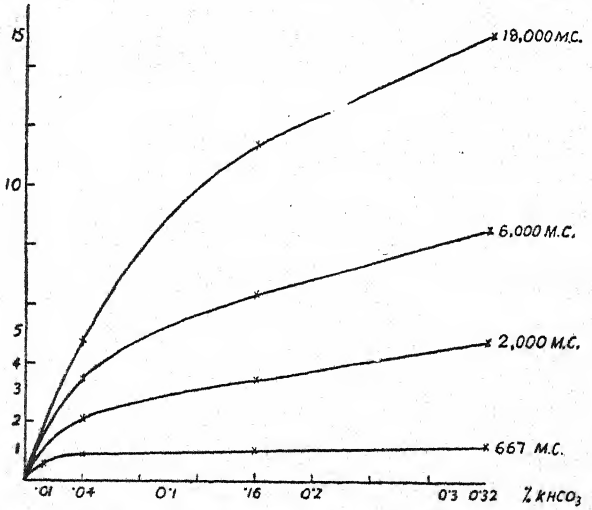


Fig. 11..—Effect of change in concentration of carbon dioxide on the rate of photosynthesis with different intensities of light (After Harder.)

fore, concludes that more than one factor may be limiting at a time and a variation in the intensity of that factor which is relatively to the greatest degree in the minimum, produces the greatest change in the photosynthetic rate. For instance, if at a certain point in the photosynthetic curve the rate of photosynthesis is augmented by either the increase in CO_2 concentration or by light intensity, greater augmentation of photosynthetic rate will take place when that factor which is relatively more in the minimum is increased.

These considerations have an important bearing on crop production. In India, both the factors of

light and temperature are high in comparison to those found in Europe. Thus for the growth of wheat, for instance, it is not the light or temperature that has to be increased for increased assimilation but carbon dioxide which relatively is in the minimum in India.

Energy Relations in Photosynthesis

In the process of photosynthesis the radiant energy is transformed into potential energy in the form of the organic compounds that are synthesised. We have then to study (*a*) the amount of energy available to the plant, and (*b*) the proportion of energy used in photosynthesis.

(*a*) The Amount of Energy Available to the Plant.

The Total Incident Radiation.—The intensity of solar radiation can be measured by means of the heat produced when the radiation is absorbed on a black surface at right angles to the rays. On an average the intensities of solar radiations vary from 1·15 to 1·75 calories per square centimeter per minute.

The Coefficient of Absorption of Radiant Energy.—The coefficient of absorption of radiant energy is the difference between the solar energy falling upon the leaf and the amount transmitted through it. This is easily estimated by interposing the leaf in the path of the rays and measuring the radiant energy falling on the leaf and the amount transmitted. The difference gives the coefficient of absorption.

This of course neglects the amount reflected and

radiated from the leaf surface. The coefficient of absorption for *Helianthus annuus* as found by Brown and Escombe was 0·686. They also measured the coefficient of absorption by the white and green portions of the leaf of *Negundo aceroides*. The result was 0·787 for the green and 0·745 for the white. Thus 0·042 was the increase in the absorptive power due to the green colouring matter of the leaf.

(*b*) The Proportion of Energy Used in Photosynthesis.

Not all the energy that is absorbed is utilized in photosynthesis. Some of it is used in transpiration while a certain portion is lost by thermal emission into the surrounding air.

One of the easiest ways to find out the amount of energy fixed in photosynthesis is by measuring the increase in weight after a period of active assimilation. This increase gives the weight of the carbohydrates formed in photosynthesis. The heat of combustion of the various carbohydrates being known, the energy fixed can be calculated out.

The following are the heat energies per gram of certain substances:—

Glucose	..	3,760 calories.
Sucrose	..	3,990 ,,
Starch	..	4,100 ,,
Cellulose	..	4,200 ,,
Leucine	..	6,500 ,,
Olive oil	..	9,510 ,,

or increase of every gram in weight of the leaves nixately 4,500 calories are fixed, for the process ot stop at glucose but substances of higher energy are also built up. From these considerations and Escombe conclude that as little as 0·5 per the total incident radiation in full sunshine is in the process of photosynthesis by land plants. tsch, however, finds that 1 to 5 per cent. of rgy is utilized.

Mechanism of Photosynthesis

DEHYDE HYPOTHESIS.

vas Baeyer in 1870 who first suggested the for- de hypothesis with regard to the formation of iate products in photosynthesis. According to to sunlight carbon dioxide splits up into carbon and oxygen. The oxygen thus formed es- and the carbon monoxide is held by the chlo- The monoxide is now reduced to formaldehyde composition of water. The formaldehyde is lensed to sugar. The successive steps are as

(1) $CO_2 \longrightarrow CO + O$ in Sunlight

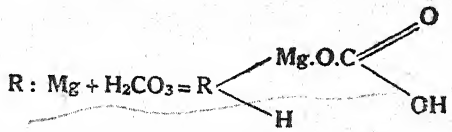
(2) $H_2O \longrightarrow H_2 + O$

(3) $CO + H_2 \longrightarrow H-C(=O)-H$

(4) $6HCHO \longrightarrow C_6H_{12}O_6$

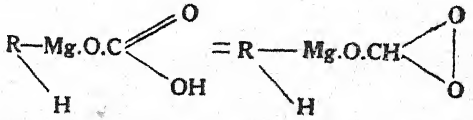
r's hypothesis in regard to the formaldehyde intermediate product in photosynthesis has far the most consideration.

Willstätter and Stoll (1918) on the results of their experimentation consider that the carbonic acid forms an additive compound with chlorophyll as follows:—

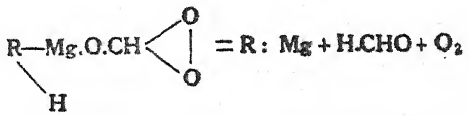


Here R:Mg stands for chlorophyll formula.

This first phase of chemical action is followed by a photochemical reaction in which the chlorophyll-carbonic acid compound gets rearranged as follows:—



This compound with a peroxide structure has a higher energy content and thus in the process absorption of light energy takes place. The peroxide now decomposes under the action of an enzyme into formal-

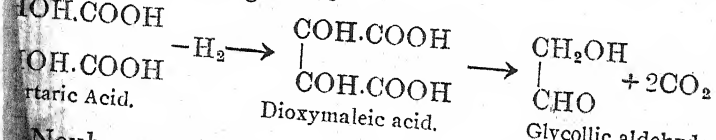


dehyde and chlorophyll is reformed. The formaldehyde now polymerises to hexose.

Organic Acid Hypothesis.

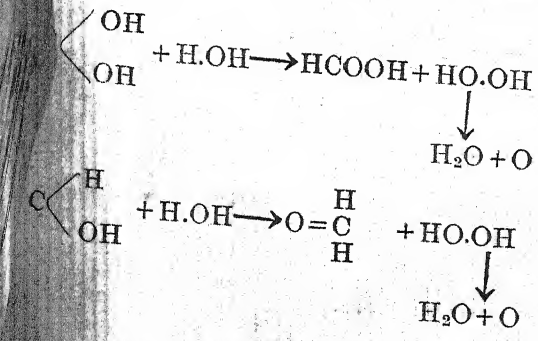
Another alternative hypothesis put forward by some workers suggests that organic acids are formed as intermediate substances during photosynthesis. Almost a hundred years ago Liebig on the basis of the decrease

in the acidity and corresponding increase in sugar in the ripening fruits came to the conclusion that acids were the intermediate products in photosynthesis. Unfortunately Liebig's experiments were not quite accurate nor could he construe at that time the oxidative respiration in plants. It has now been shown that in succulents the acids are broken by photolysis in light to liberate carbon dioxide which then is used in photosynthesis. Thus the acids decrease in the day and increase during the night. Fenton has shown that Tartaric acid when treated with hydrogenperoxide and ferric sulphate yields dioxymaleic acid which on warming in water solution looses carbon dioxide and yields glycollic aldehyde. The latter is easily converted to glucose



Neuberg and Schwenk have shown that by the of yeast dioxymaleic acid gets converted to glyaldehyde.

Erlenmeyer, however suggests the following



In this scheme hydrogenperoxide is formed which will be reduced to water and oxygen by the action of the enzyme Catalase. That Catalase increases during the formation of monosaccharides has been shown by Ranjan and Mallik (1931). This supports Erlenmeyer's hypothesis because during active synthesis of sugars large quantities of peroxide are formed which get decomposed at a very rapid pace: in fact at the very moment of formation. Thus proportionate increase in the amount of catalase is important.

Practical Experiments

FACTOR CO_2.

1. A potted plant is placed on a ground glass plate and is covered by a tubulated bell-jar. Inside the bell-

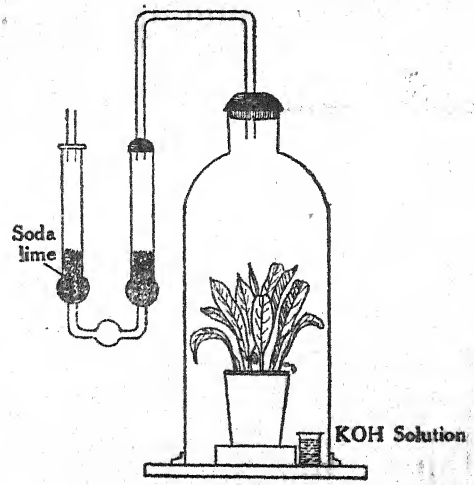


Fig. 12.—Diagram of experiment to show that photosynthesis cannot take place in absence of CO_2.

jar is also put a dish containing strong solution of caustic soda. The edge of the bell-jar is sealed air-tight by means of vaseline to the ground glass plate. The aperture of the bell-jar is closed by a rubber stopper through which a tube containing soda lime communicates (Fig. 12). The apparatus is placed in bright light for some hours. If the leaves are now tested for starch it will be found that they are without starch.

2. *Moll's half-leaf experiment.*—Two crystallizing dishes whose well ground edges fit closely when put

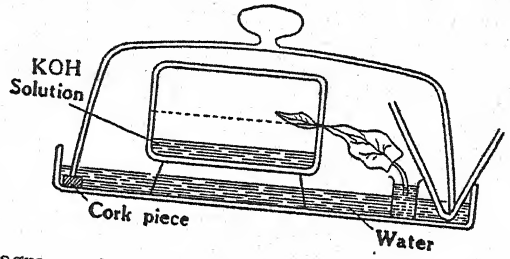


Fig. 13.—Diagram of Moll's half-leaf experiment to demonstrate the importance of CO_2 in photosynthesis.

together are taken. Starch free leaf of *Arum,* taken from a plant which has been kept in the dark for some time so that it is starch free, is placed between the edges in such a way that the tip of the leaf is within the closed space, the base of the leaf and the leaf stalk being outside. The lower dish contains potash solution. The leaf petiole dips into a small vessel containing water. The whole apparatus is now placed under a large calibrated bell jar, which rests on cork pieces and dips in water. By means of the bent tube some water is sucked out and pure CO_2 led in to replace the quantity of water (Fig. 13). The apparatus is exposed for some hours to sunlight. It is found that the portion of the leaf kept in air is rich in starch while no starch is present in the terminal part of the leaf.

3. *Bubbling of oxygen.*—A number of shoots of *Hydrilla* (a water plant) are placed under a funnel in

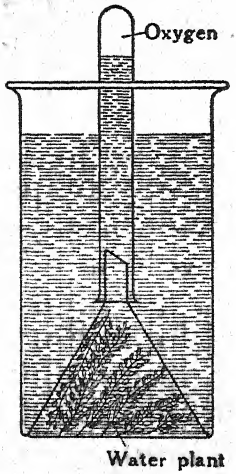


Fig. 14.—Diagram of apparatus to show the evolution of oxygen by water plants during photosynthesis.

a large beaker filled with water holding carbon dioxide in solution. Over the end of the tube of the funnel which is below the surface of water is inverted a test tube filled with water and the whole exposed to direct sunlight (Fig. 14). Oxygen collects in the test tube.

4. *No bubbling of oxygen without* CO_2.—The above experiment is repeated by putting the *Hydrilla* plant in a beaker containing water which has been previously boiled and kept covered with a view to remove the CO_2. No bubbles appear.

5. *Experiment to show that the* CO_2 *enters the leaf through stomata.*—The lower half of a leaf without being cut off from the plant is smeared with vaseline. After 24 hours the leaf is removed from the plant and its chlorophyll is extracted by first boiling in water and then in alcohol. It is then submitted to the iodine test.

The portion of the leaf whose stomata were sealed forms no starch, while the other portion forms starch.

FACTOR LIGHT.

1. A green leaf from a plant growing in light is boiled first with water and then in alcohol to remove all the pigments. It is then put in dilute iodine-potassium iodide solution. Note the dark blue colour of the leaf showing the presence of starch grains formed as a result of photosynthesis.

2. Same experiment as above is repeated but with a leaf of plant kept in darkness for 48 hours. Note that no colouration develops in iodine solution, showing the absence of starch grains in the leaf. Here the starch already present in the leaf was hydrolised and translocated to other parts of the plant when it was kept in darkness and no fresh starch could be formed.

3. A piece of card-board in which the letter H is cut off is clamped 24 hours on a leaf which is kept in the sun. It is then removed from the plant, decolourized and put in iodine solution as above. A dark prominent H is formed in a clear background. Explain.

4. Two double walled bell-jars are filled with dilute solutions of potassium dichromate and copper sulphate respectively. *Hydrilla* plants are placed within these bell-jars in two beakers covered with a funnel and an inverted test tube filled with water. These are placed in the sun. Note that the oxygen collected in the test tube inside the orange coloured bell-jar is greater than the one in the blue coloured bell-jar.

FACTOR CHLOROPHYLL.

1. Variegated leaves of *Croton* and *Arundo* are taken and their chlorophyll is extracted. Then they

are put in iodine solution. Note that the dark colouration appears only at the regions containing chlorophyll. Explain.

2. With the help of a hand spectroscope, examine the light spectrum:—

(*a*) Direct, and

(*b*) Through a solution of chlorophyll.

Note that in the latter case a dark band appears in the red region and another in the blue-violet region.

3. *Sun and shade leaves.*—Cut T.S. of the leaves of *Porana paniculata* or *Nasturtium* growing in sun and shade respectively. Note that the two kinds of leaves differ in the following ways:—

(*a*) Number of palisade paranchyma layers—larger in sun leaves.

(*b*) Number of stomata—more in sun leaves.

(*c*) Number of chloroplasts in each palisade cell—greater in sun leaves.

(*d*) Position of the chloroplasts—central in shade and nearer the cell walls in the sun leaves.

Examine the sections under the microscope. Sketch and show the various differences and explain.

4. Mount a few filaments of an alga and note the position of the chloroplasts. Treat the alga with chloral-hydrate-iodine and observe that starch grains are found at places occupied by the chloroplasts.

5. Mount the leaf of a moss plant and treat it also with chloral-hydrate-iodine and note the result.

6. Cut T.S. of the leaves of *Nerium* and *Eugenia* and treat the sections as above with chloral-hydrate iodine and note the result.

Movements of Gases in Plants.

1. The apparatus (Fig. 15A) is closed with a cork in which a leaf-stalk is cemented air-tight. A glass cylinder containing water is fitted air-tight on the top of the apparatus. The leaf petiole lies just below the surface of water. Mercury is now poured into the side tube to compress the air, which enters the stomata of the leaf and escapes at the cut surface of the petiole.

2. The mouth of one side of a glass cylinder is closed with a cork in which a leaf-stalk is cemented air-tight. The cylinder is now half-filled with water. The other end of the cylinder is also closed with a cork in which a hole is bored to allow a glass tubing which is connected to a suction pump by a rubber tubing. The suction pump is now allowed to work. Bubbles are seen coming out of the surface of the petiole (Fig. 15B). Explain.

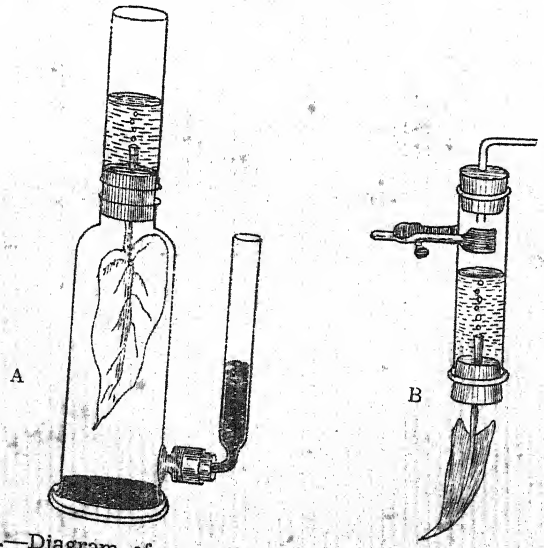


Fig. 15.—Diagram of arrangements to show that inter-cellular spaces of plant tissues are in communication with the atmosphere.

CHEMOSYNTHESIS OR THE ASSIMILATION OF CARBON BY PLANTS WITHOUT CHLOROPHYLL

So far the formation of the carbohydrates in the green cells of the leaf has been dealt with. For this endothermic reaction the energy is derived from sunlight. There are, however, certain forms of bacteria that grow in the entire absence of light and still form their carbohydrates from carbon dioxide and water. They derive their energy for this process from ammonia and such other compounds.

So that there are two sources of energy for plant activities, *viz.*,

(I) Sunlight, and

(II) Energy from oxidation of substances.

The latter can further be sub-divided into:—

(1) Those that derive energy from organic compounds, and

(2) Those that derive energy from inorganic compounds.

1. The non-green plants that derive energy from organic compounds are further divided into:—

(*a*) Saprophytes .. (Mushrooms, yeasts, etc.)

(*b*) Parasites .. (*Cuscuta*, etc.)

2. Non-green plants that derive their energy from inorganic substances are as follows:—

(*a*) Nitrifying bacteria—There are two types of bacteria found in this class, *viz.*,

The *Nitrosomonas* which oxidises ammonia to nitrous acid and *Nitrobacter* which further oxidises the nitrous acid to nitric acid. This oxidation provides these micro-organisms with the necessary energy for sugar formation. The reactions are as follows:—

Nitrosomonas— $(NH_4)_2CO_3 + 3O_2 \longrightarrow 2HNO_2 + CO_2 + 3H_2O + 148$ cal.

Nitrobacter— $Ca(NO_2)_2 + O_2 \longrightarrow Ca(NO_3)_2 + 22$ cal.

(*b*) Sulphur bacteria is another example of non-green plants which derive their energy from inorganic sources. They oxidise hydrogen sulphide to sulphur and water, thus securing the energy for metabolic processes.

The sulphur bacterium, *Beggiatoa*, is found in sulphur springs and thrives on putrefying material which produces sulphuretted hydrogen. The reactions are as follows:—

$$2H_2S + O_2 \longrightarrow 2H_2O + S_2$$

The sulphur so liberated accumulates in the protoplasm, and gets further oxidized to sulphates:—

$$S_2 + 2H_2O + 3O_2 \longrightarrow 2H_2SO_4.$$

Sulphuric acid immediately reacts with the carbonates present in the water to form sulphates.

$$H_2SO_4 + CaCO_3 \longrightarrow CaSO_4 + CO_2 + H_2O$$

Regarding the non-green plants that derive energy from organic compounds only, mushroom and yeast have already been mentioned in connection with saprophytes and are dealt with in books dealing with fungi.

Cuscuta (dodder) is an example of the parasitic kind. It is parasitic on most green plants. This

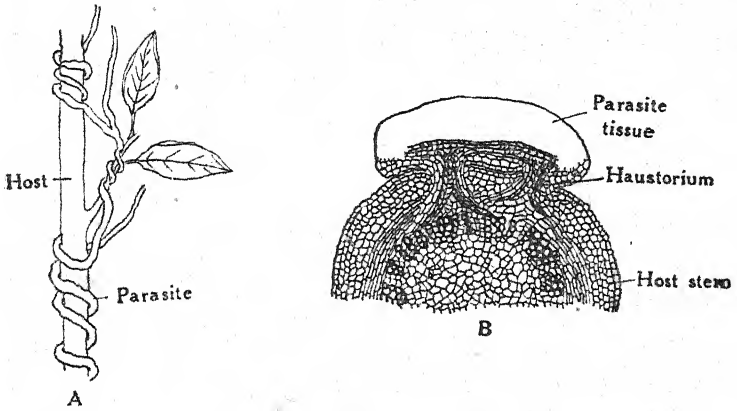


Fig. 16.—(A) *Cuscuta* twining round a host; (B) Cross section of host with attached parasite. Note the vascular connection between the host and the prarasite through the haustorium.

is an extreme case of specialization. For the entire plant body resembles a fungus. It consists of leafless branches devoid of chlorophyll which may be compared to a fungus hypha. The hypha like body attaches itself by means of its haustorium to the host. Vascular connections from the bundles then develop and reach the vascular bundles of the host, to enable the parasite to draw upon the nourishment of the host (Fig. 16).

NITROGEN SYNTHESIS

Our study of plant life has shown us that, as a rule, green plants and many fungi and bacteria, form amino-compounds, proteins and other nitrogenous

bodies from certain of the raw materials. The question now arises as to the sources of the nitrogen.

Naturally attention is drawn towards the atmospheric nitrogen which is about four-fifth of the air around us. Is this uncombined element assimilated by plants in a somewhat similar way to carbon dioxide assimilation? In his classic experiment Boussingault proved that the majority of plants are unable to assimilate atmospheric nitrogen.

He germinated a seed in soil, free from nitrogenous compounds, in a pot. The seedling was allowed to grow inside a bell jar, through which air along with nitrogen was allowed to circulate.

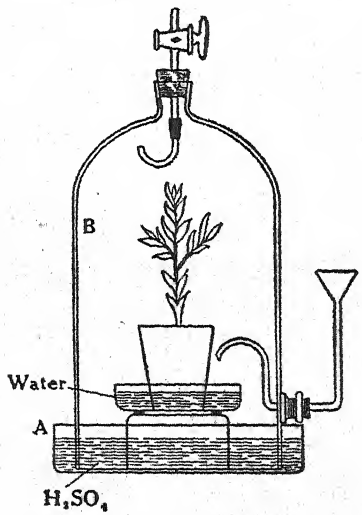


Fig. 17.—Diagram of Boussingault's experiment (For explanation see page 72).

Any ammonia in the atmosphere was absorbed by sulphuric acid kept in a dish within the bell jar.

Boussingault found that though carbohydrates increased the total nitrogen remained the same as it was in the seed. More extensive and critical experiments have since been performed and they have all gone to confirm this result. One family of plants, however, stands out unique and that family is the Leguminosae. Plants of this family are able to trap the atmospheric nitrogen and build up complex proteins. Their mode of nitrogen nutrition will be dealt with later.

Having thus found atmospheric nitrogen to be of no use to the vast majority of plants we have to turn our attention to soil nitrogen. Nitrogen occurs in soils in various combinations, *viz.*,

(1) Undecomposed organic matter.

(2) The converted organic matter due to decay —humus.

(3) The inorganic nitrogen in the form of nitrates and ammonium salts.

Nitrification of the Soil.

Predominantly nitrification of the soil is brought about by bacterial action. If a large bored glass tube about a yard long is taken and is filled with a mixture of sand and lime, and sewage water containing ammonia is poured from top, so as to allow it to slowly percolate through the mixture of sand and lime, then, after some days, nitrates will be found. The ammonia of the sewage gets oxidized to nitrates by the action of bacteria present in the sewage water. If chloroform

is added to the water then the formation of nitrates stops.

In the process of decay and putrefaction (the former process is odourless and the latter with odour) of plants and animals ammonia, carbon dioxide and other products are produced. These processes are brought about by *Bacterium mycoides.* Some of this ammonia escapes into the atmosphere, while some unites with the soil bases and remains in the soil. The ammonia is further oxidised by certain micro-organisms to nitrites. These nitrites are, however, injurious and are converted by other types of bacteria to nitrates. The nitrates are then, in their turn, taken up by the plants.

The Relative Importance of Nitrates and Ammonia.

In all ordinary cases nitrates and ammonia are present in the soil moisture as ions and enter the root

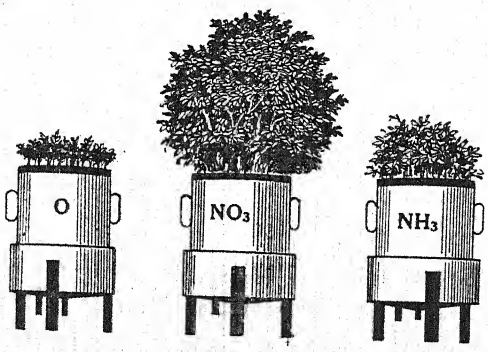


Fig. 18.—Experiment showing the relative growth of plants when supplied with ammonium and nitrate nitrogen (after Wagner).

hairs as such. Regarding the utility of these two forms of nitrogen it has been found that in some cases plants prefer nitrate nitrogen while others prefer ammonium nitrogen.

When ammonium salts are supplied to the soil it is just possible that they get first converted into nitrites and then into nitrates and as such enter the root hairs. If soils do not contain lime then it has been shown that there is more growth when nitrates are supplied than when ammonium salts are given (Fig. 18). The reasons are twofold, *viz.*,

(1) When ammonia is oxidised to nitrates then free nitric acid may result which increases the acidity of the soil and is consequently injurious. If, however, lime is present the nitric acid will combine with calcium to form calcium salts.

(2) If ammonia is assimilated as such in the form of ions then more of the basic radical is absorbed and the acid ions are left behind. This again tends to increase the acidity of the soil.

Nitrogen Fixation.

By nitrification nitrogenous bodies are merely transformed into inorganic nitrogen. But this does not increase the total nitrogen of the soil. No doubt some combined nitrogen may be brought down by thunderstorm, but it is in negligible amounts. On the other hand there is a continuous loss of nitrogen by the washing away of sewage and by denitrification by certain bacteria.

This loss of nitrogen is made good by plants belonging to the family Leguminosae, which by a peculiar process are enabled to fix atmospheric nitrogen. Legumes growing under natural conditions have small tubercles upon their roots. These develop only when grown in non-sterilized soil.

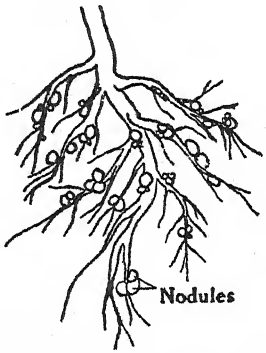


Fig. 19.—Root of a leguminous plant with tubercles.

A transverse section of a leguminous root shows that the inner cells of the parenchymatius tissue are different from the outer parenchymatous tissue. The inner one is the bacterioid tissue.

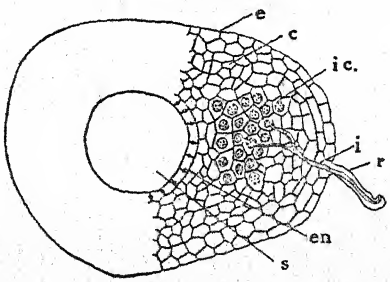


Fig. 20.—Cross section of a young root nodule: c, cortex; e, epidermis; en, endodermis; ic, infected region of the cortex; i, infection thread; s, stele and r, root hair through which bacteria entered.

The cells of this tissue have high protein content. The protein substances occur as small bacteria like rods. Strands of vascular bundles of the root also extend towards the tubercles in older nodules.

Life history of Bacillus radicicola.—The bacterium which lives symbiotically in the roots of the Leguminosae is *Bacillus radicicola.* This in some mysterious way has the power to take up the atmospheric nitrogen present in the soil and convert it into organic nitrogen compounds. It, however, cannot manufacture its carbohydrates and thus has to depend for this commodity upon the leguminous plant to which it gives back in return organic proteins. This is a typical case of interdependence or symbiosis.

The bacteria which at first are in the form of spherical small bodies with two cilia, remain in the ground and when they come near a root hair get attached to its mucilagenous wall. Soon after they manage to penetrate in and accumulate within the root hair. Later they get enclosed in a sheath, which enlarges and branches, and penetrates into the root paranchyma. The filamentous sheath then begins to branch again. At the same time, due to the irritation by the presence of a foreign body, rapid division of the root parenchyma also takes place. This gives rise to the tubercle. The filamentous sheath now disintegrates liberating the bacteria. Here they enlarge and branch and become bacterioids. At this time vascular bundles develop in the tubercle and deplete the bacterioid tissue. The remaining bacterial cells col-

lect in groups and get enclosed in strong sheaths. These spore-like colonies fall away on the death of the host and infect other roots.

Protein Assimilation.

The lack of knowledge regarding the chemistry of the proteins makes it impossible to study with any definiteness their synthetic process. As has been said before, the proteins are an immense group of compounds with enormous molecules. They contain carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. When the higher proteins are hydrolysed they yield amino acids. These are the products which are intermediate between organic acids or carbohydrates and proteins.

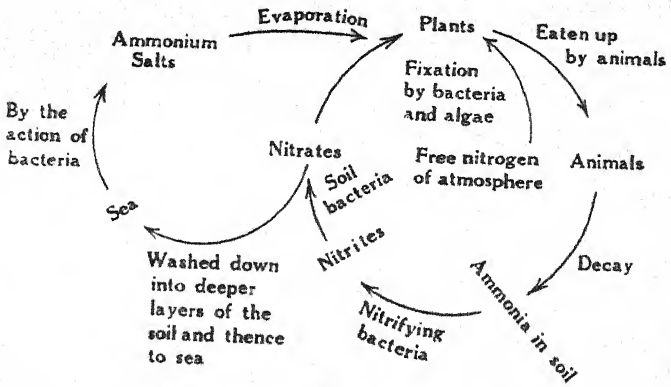
Though theoretically speaking a large variety of amino acids as a result of hydrolysis of proteins should be formed, recent work has shown that about twenty different types of amino acids only are found in the leaves of the plants in various proportions. On the other hand at the meristematic regions where young protoplasm is being formed there is preponderance of Glutamic acid and Aspartic acid. The fewer types of amino acids leads one to conclude that the metabolic activities of the proteins are canalised in definite channels according to the protoplasmic structure of each group of plants.

It has been shown by Baly that when in an aquous solution of potassium nitrite or potassium nitrate, carbon dioxide is bubbled in the presence of ultraviolet

light, then an amino acid called glycine of the formula CH_2NH_2COOH is produced. From the condensation of glycine higher proteins can be formed.

Within the plant proteins are formed in the leaves in light, though they have been shown to be formed in darkness also. As the formation of the proteins from nitrites, or nitrates involves the fixing of energy, this has got to be supplied and the energy relieved from the breakdown of the carbohydrates in respiration is believed to be used in protein synthesis. In fact it has been shown that when leaves are floated on sugar solution and a solution of potassium nitrate and kept in the dark then also sufficient quantity of proteins are synthesised.

Circulation of Nitrogen in Nature



Practical Experiments

1. Examine the root nodules in *Lathyrus*. Make a drawing of the same. Bacteria are always present in the soil and infect the roots of leguminous plants through the root-hairs. In the root-hairs they multiply

and make their way into the cortical tissue and stimulate it to active growth to form the tubercles. The tubercles are very rich in nitrogenous substances and seem to develop in soils poor in nitrogen compounds.

2. These one of the nodules and examine the small bacteria under the microscope.

3. Examine the total parasite *Cuscuta* (Akashbel) on the host. The flowers of this plant are quite normal but not the vegetative growth. The seedling sends a short root into the ground while its shoot elongates rapidly and nutates vigorously. When it meets a suitable host, it claps round it and sends out haustoria which eat their way through to the vascular bundles of the host plant where the xylem and phloem of the parasite fuse with the corresponding tissues of the host plant; and thus it obtains its supplies of organic food as well as of water and salts dissolved therein. Meanwhile the root of the parasite dies off and the plant becomes independent of the soil. The plant possesses no leaves other than small scales. The stem is not green.

Cut T.S. of *Cuscuta* through its attachment to the host. Stain, examine and sketch.

4. *Boussingault's experiment to demonstrate that free nitrogen of the atmosphere is not directly assimilated by a green plant.*—The apparatus used in this experiment is shown in Fig. 17.—It consists of a trough A. Inside this trough is inverted a bell-jar B, which is raised from the floor of the trough A, by being rested on corks at various places. Inside the bell-jar is another trough, much smaller in size, and a flower-pot is kept in this trough. The trough A is filled with diluted sulphuric acid, and the smaller trough with water.

Previously the total nitrogen of the seeds of some non-leguminous plant is estimated. A little of the ash formed during the above chemical analysis is added to

previously sterilised sand to prevent the plant from dying early. The amount of nitrogen in this ash is carefully noted before the seedling is germinated. This soil is put in a pot and a seed of the same non-leguminous plant is sown in it, and this pot is kept under the bell-jar as mentioned above. Two tubes pass inside, one is connected with the trough (smaller) to pour in water when needed, through the other carbon dioxide is allowed to pass inside the bell-jar.

The dilute sulphuric acid is to absorb traces of ammonia, if any, in the atmosphere. The plant is now surrounded by free nitrogen of the atmosphere. After some growth the plant is carefully burnt and the total nitrogen in the ash is estimated. It is seen that there is no increase in the amount of nitrogen, beyond the amount already present in a similar seed. This shows that free nitrogen is not assimilated by the green plant.

INSECTIVOROUS PLANTS

The fact that animals eat and digest plants is of common knowledge. But the reverse case of plants catching animals and actually digesting them is rare. Nevertheless such plants are found in nature though they prey upon only small insects. Stories about big trees found in African jungles that catch and devour large-sized animals are pure myth: such plants have never been seen.

The insectivorous plants are grouped together in five rather artificial families. Their evolutionary history shows that the evolution has proceeded on several lines.

The five families are as follows:—

(1) Droseraceae—to this belong the plant *Drosera* and the Southern American *Dionaea muscipula*, also called the Venus' fly-trap.

(2) Nepenthaceae—with the single genus *Nepenthes*.

(3) Sarraceniaceae—belonging to the tropical and sub-tropical America.

(4) Cephalotaceae—with the single species *Cephalotus follicularis* growing in Australia.

(5) Lentibulariaceae—to this belong the common *Utricularia* and *Pinguicula*.

The first four families belong to the Archichlamydeae and the fifth to the Sympetalae.

As their name signifies the insectivorous plants have arrangements for catching insects. These, then, by suitable enzymes secreted by the plants are digested and absorbed.

The leaves of all the insectivorous plants contain chlorophyll. So that they are able to manufacture their own food materials like the other autotrophic plants. What is then the necessity of the insectivorous habit? It certainly cannot be for carbon assimilation, but it may be for nitrogen assimilation. It has been noticed that when these plants are moderately supplied with insects, they grow more healthy and produce more fruits and seeds. It is evident then, that the organic form of nitrogen is eminently suitable.

As a rule these plants grow in boggy places and have very poorly developed root system.

1. *Drosceraceae.*—In this family there are five genera and about a hundred species but ninety of them belong to the genus *Drosera.* In popular language they are called sundews. Found chiefly in Australia. the family is as a rule cosmopolitan. In India, they have been found plentiful in the Chittagong District and also in some parts of Madras Presidency.

These are small plants of wet peaty moors. Their leaves form a rosette from which arise flowering stems bearing cymes of white flowers. The leaves having a reddish hue, give the appearance from a distance, of a scarlet felt against the green background. Roots are poorly developed. The leaves are stalked and the leaf blades are beset with numerous tentacles.

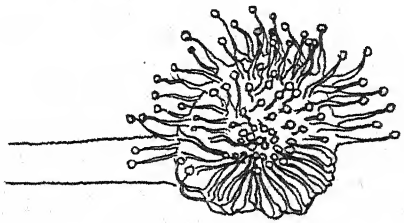


Fig. 21.—Diagram of a leaf of **Drosera.** Tentacles of a part of the leaf shown bending to entangle insects.

The stalk of the tentacle is traversed by tracheids which end in the club-like head of the stalk in a massive formation. The tracheid head is surrounded by three layers of cells. The outer being secretory is filled with a red sap while the inner is the bundle sheath. The

marginal tentacles are longer than the central ones. These tentacles are situated only on the upper surface of the leaves. The globoid heads of the tentacles are covered up by a sticky substance secreted by them. When the insect touches the tentacles, it sticks on the sticky spherical head. The glands now get active, and begin to pour out copiously a sticky fluid. At the same time the marginal tentacles get affected and they bend towards the centre. Thus the insect gets covered over both by the tentacles and their copious secretions.

The secretion contains the enzyme pepsin and an acid. The pepsin acts on proteins of the insect and converts them into peptones. The process of digestion takes several days. When completed the tentacles resume their original position.

2. *Nepenthaceae.*—To this family belongs the single Genus *Nepenthes,* with about fifty species. It is found abundantly in Malaya Archipelago. These are small shrubs, epipytes or climbers. Some may attain a length of ninety feet. It is also known as the *pitcher plant.* The pitcher is a portion of a leaf blade. The broad sheathing blade first runs into a tendril. After making a round through some support, it bends vertically down and then curves up terminating in the characteristic pitcher. The pitchers often have brilliant colours, *e.g.,* red and purple. They thus are modified parts of the leaf tips. Not all leaves form pitchers.

The edge of the pitcher has a rounded ribbed rim of vascular bundles which keeps the circular edge taut. Thus the mouth of the pitcher is always open.

The structure inside the pitcher, can be divided into two portions, *viz.*, (1) the upper waxy portion and (2) the lower glandular portion.

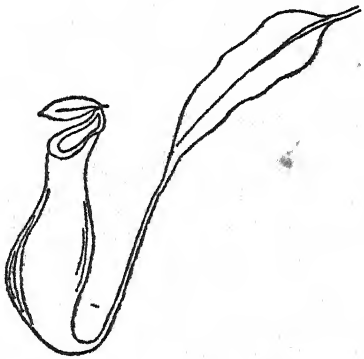


Fig. 22.—A pitcher of **Nepenthes.**

The upper cells in the neck are smooth cells, forming papillae. These are covered with slippery wax scales which are modified abortive guard cells and project downwards. The lower glandular portion is multicellular and are half sunk in epidermal cavities and secrete a watery fluid which is found in the pitcher.

Glands are also found on the under side of the lid as also on the rim. These secrete nectar which attracts the insects.

Knoll demonstrating the slippery nature of the upper zone used wingless ants, which possess curved claws with which they walk on rough surfaces, and adhesive cushions with which they can climb on perfectly smooth glass plates. Both these were found

to be useless to the ant because the smooth downwards projecting papillae gave no hold while the adhesive cushions though they gave a grip to the waxy scales were found to be useless as the scales themselves slide down due to the weight of the insect.

The liquid in the pitcher, before any insect reaches, is neutral in reaction and contains no digestive enzymes. But the introduction of an insect produces lot of internal secretions containing formic acid and proteolytic enzymes. The liquid is antiseptic because of the acidity; thus there is no growth of bacteria.

3. *Sarraceniaceae.*—The plants of this family are natives of tropical and sub-tropical America. There are 3 genera and about 10 species.

Fig. 23.—A pitcher of Sarracenia.

In this, unlike the previous family, the whole of the leaf is transformed into a pitcher. The leaves grow direct from the soil and are arranged in rosettes. Only the maturer ones, however, form the pitchers.

The outer side of the rim is prolonged into an arched lid which partially protects the mouth. About the rim lot of nectar is secreted. Just below the rim is the slippery zone. Here the smooth cuticular epi-

dermal cells have their lower edges projecting over the cells next below. This gives the appearance of a tiled roof. The zone below this bears long bristles pointing downwards. Nectar glands occur here and there.

The insect attracted by the bright colour alights on the rim and crawls around the margin to get the nectar just inside it. In doing so it slips and quickly disappears inside the pitcher never to come out again.

4. *Cephalotaceae.*—This pitcher plant of western Australia is little investigated. It is represented by a single species *Cephalotus follicularis.*

The leaves are in rosette and arranged in two tiers. The upper is normal and flat while the lower forms pitchers. The pitchers are short and flat and have the usual ribbed rim. There is a double wing running down the entire length of the pitcher while single wing-like projections run down each side of the pitcher. The mouth is covered by an arched lid. Within the pitcher slippery zones as also glands can be found.

5. *Lentibulariaceae.*—This belongs to the great group Sympetalae. There are 5 genera and 300 species in this family. The type *Utricularia*—commonly called the bladder-wort—is found mostly, in the tropical regions, in water. There are also certain land forms.

Except the flowering axis which is above the level of the water, the rest of the plant is submerged. Vegetative part consists of a much divided shoot bearing a number of much divided leaves. Many of the ulti-

mate segments of these are replaced by bladders. Roots are entirely absent.

The bladder is a small oval structure about one-tenth of an inch long and is attached by a stalk to the

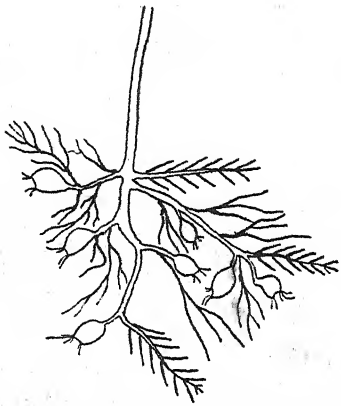


Fig. 24.—**Utricularia,** leaves with bladders.

leaf. At the pointed end of the bladder there is an opening which is generally closed by a valve-like lid joined to the margin above. The free lower end fits firmly on the thickened rim of the opening. From the outer surface of this valve arise a few long branched hairs. Also on the surface of the bladder, frequently hair-like appendages are borne.

The Mechanism of the Opening of the Bladder.— According to Darwin and other earlier workers this analogous mouse trap has a passive check valve easily pushed inwards, and not outwards. The prey once caught cannot come out. According to the present day ideas of the Utricularia trap, the mechanism, as depict-

ed in a very interesting way by Heath Robinson, is active and complex including:—

(i) Mechanical devices as if involving the use of hinges, pulleys, etc. As a result of these the door is opened and the entrance of the prey is ensured.

(ii) Electric devices, which may be likened to the use of a motor which is set in action after the prey has entered in and acts upon various springs etc., causing ultimately the door to close.

Recently Czaja has shown that the bladder walls are impermeable to water. On the inner walls are situated

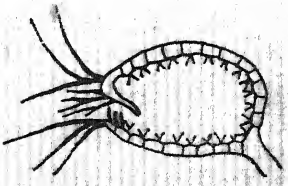


Fig. 25.—A bladder of **Utricularia** in longitudinal section.

numerous hairs and these withdraw water from inside the bladder causing partial vacuum. The two side walls are thus pulled in and may show dimples on the two sides. Hence a considerable tension is set up. If now an insect touches one of the hairs on the outside, the valve is slightly opened and the water with the insect rushes in. The valve now closes making the escape of the insect impossible. Thus a pricked bladder will not function.

No digestive enzyme has yet been found in *Utricularia*. Probably the enclosed insects when they die

are disintegrated by bacterial action, after which they get absorbed by the plant.

Practical Experiments

Carnivorous or Insectivorous Plants.

These plants obtain part of their nitrogenous food by catching insects in various ways. Among the Indian insectivorous plants are *Pinguicula, Drosera, Utricularia* and *Nepenthes.*

1. *Pinguicula* (Butterwort).—It is occasionally met with in the alpine Himalayas. The plant has a basal rosette of broad leaves, whose upper surfaces are covered with sticky glands, while the margins are rolled inwards slightly. Small insects are caught by the sticky secretion and washed by rain to the edge of the leaf, which curls inwards and encloses them; the glands secrete digestive ferments. The digested products are absorbed and then the leaf unrolls again.

2. *Drosera* (Sundew).—Common in India. It belongs to the family Droseraceae. The leaves of this plant bear numerous stalked glands or tentacles which secrete a sticky fluid. If an insect adheres to the tentacles they bend down upon it, and pour out a fluid which has the power of digesting it. The secreted fluid is reabsorbed together with the soluble nitrogenous products. When digestion is complete the tentacles resume their former position.

3. *Dionaea* (the Venus' fly-trap).—This is a native of California, where it grows in peat-bogs. The leaves are two lobed and the midrib acts as a hinge. Each lobe bears on its upper surface three long sensitive hairs. When one of these is touched by an insect, the two lobes of the leaf snap together and capture the insect. Digestion occurs as in *Drosera.*

4. *Utricularia* (Bladderwort).—This is a submerged water plant which has no roots. The submerged parts bear curious bladders, each with a trap-door or valve, which is easily opened by a push from the outside, so that small animals (insects, water-mites, water-fleas etc.) cannot escape once they have entered the bladder. When these animals die, their soft parts decay and are absorbed by branched hairs which occur on the inner surface of the bladder.

5. *Nepenthes.*—This is known as a pitcher plant. A part of the leaf is developed as a pitcher with a lid attached to one side of the opening. The bottom of the pitcher contains water, and a digestive fluid. When any insect falls in the fluid, it is first drowned and then digested.

6. *Sarracenia.*—It is another pitcher plant found exclusively in America. In this plant there is no ferment. The bodies of the insects are decomposed by the action of bacteria and the soluble products absorbed.

SYNTHESIS OF FATS

That fats occur in most green leaves during some stage of their life history is now a well established fact.

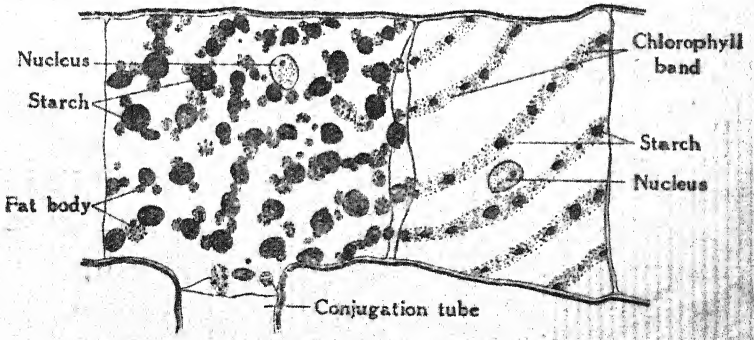
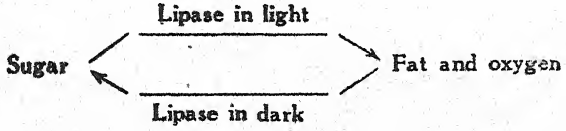


Fig. 26.—**Spirogyra** cells with oil globules (after Pal).

The fats are often known as oils when present in the liquid state. An examination of an ether extract of the leaf tissue of cabbage shows abundance of fat. Leaves of *Eugenia jambolana* when young have no oil in them, but towards senescence an abundance of oil globules are visible in the spongy parenchyma.

In the case of *Spirogyra* also no oil is found in the vegetative period of their life but abundance of oil is visible at the time of conjugation. Light is essential for the formation of oil at this stage.

Although the oils are quite different chemically from the carbohydrates, there is an intimate and significant physiological relationship between these compounds in the plant. In *Spirogyra* it is found that at the sexual stage, in light, the carbohydrates get converted to oils and the reverse process takes place in darkness. The scheme of reactions is as follows:—



Leclerc due Sablon (1896) while studying the development of the kernels of walnut and almond found that as the sugar content of the developing kernels diminished, the amount of oils correspondingly increased, and when the oil content had reached its maximum, glucose had completely disappeared.

Garner, Allard and Foubert (1914), found a very sharp increase in the percentage of oil in the soyabean

during the first few weeks after blooming and a slow gain until near the end of the ripening period.

As was mentioned in a previous page, fats are condensation products of glycerol and fatty acids. It is assumed that at some stage of synthesis of fats, glycerol and fatty acids must be formed; the former either as a cleavage product of carbohydrates or during the early stages of glucose formation and the latter, i.e., fatty acids through some simpler compounds such as acetaldehydes, the condensation of the two giving the fat.

The fats are of higher energy content than the carbohydrates and, therefore, in the formation of fats from carbohydrates, a great quantity of energy is bound up.

The synthesis of fats and their formation from carbohydrates can be shown as follows:

Outside the living cell, however, the transformation of carbohydrates into fats or *vice versa* has been accomplished only to a slight degree.

Dunlop and Gilbert (1911) mixed glycerol, oleic acid and oilfree castor bean powder and after some days obtained evidence of the synthesis of oil. Thus they showed that the synthetic reactions are catalyzed by the plant enzymes and are not the function of the protoplasm itself.

Practical Experiments

1. Mount a few filaments of *Spirogyra* and stain with Sudan III. Note the absence of fats.

2. Mount the filaments of *Spirogyra* when they are in the process of conjugation and stain with Sudan III. Oil is found to be present.

3. Stain some conjugating *Spirogyra* filaments which have been kept in darkness for about 48 hours with Sudan III. It will be seen that no oil is present.

From the above experiment the following conclusions are drawn:—

(*a*) No oil is present in the *Spirogyra* in its vegetative stage.

(*b*) Oil is formed at the time of conjugation.

(*c*) Light is necessary for the formation of fats and oils.

4. Cut a T. S. of a fully mature *Eugenia jambolana* leaf, just turning brown. Stain with Sudan III and examine under the microscope. Oil globules will be seen. Sketch.

5. Cut a T. S. of a Cabbage leaf, stain with Sudan III, examine under the microscope for oil globules and Sketch.

CHAPTER V

RESPIRATION AND FERMENTATION

Respiration as an Oxidative Process

The essence of life is change which means every living cell is undergoing a change, however small, every moment. Each cell of a resting seed is every moment undergoing a change, but it is so small that it is well nigh impossible to detect it. On the other hand, inside an active meristematic cell violent changes may be going on. To bring about any change of state energy is needed, for without energy inertia will set in. Thus only in a dead cell where the internal changes have ceased, no energy is utilized.

All oxidation processes being exothermal give out energy. One must remember, however, that oxidation and reduction may not occur simultaneously at a particular spot: oxidation may be taking place at one time and one place in a cell while reduction may be going on at another time and place.

Respiration is one great group of oxidative processes, and *fermentation* is another specialised group of oxidative processes. Both respiration and fermentation are oxidative processes that serve metabolism.

Respiration may be defined as an oxidative process that goes on in every living cell whereby energy is released. The reason why respiration is normally considered as an oxidative process is because the final pro-

ducts (carbon dioxide and water) are the same as are derived by the combustion of organic substances in air. The most striking thing about respiration is that the oxidation process takes place at ordinary room temperatures while the oxidation processes outside a living cell require very high temperatures.

Sources of Energy

The organic substances produced by plants are the chief sources of energy. As has been discussed in detail in the previous chapter, the food manufactured by the plant contains the potential form of the radiant energy trapped by the green leaves. On oxidation this energy is liberated and assumes the form of kinetic energy which can then be utilized for work by the organism. One can compare the plant to a factory, where coal is necessary to work the engines. The coal is nothing else but a portion of a plant of a bygone age, which had trapped the solar energy in its day. It then, somehow, got buried underneath the earth's surface and got carbonised. This coal with its potential energy stored within it is oxidized to give out the kinetic energy to run the engines of a factory. The difference between a factory and a plant, however, is that in the former the potential energy has to be supplied to it, while the latter traps it itself.

De Saussure was the first to say that as in animals, respiration goes on in plants as well. But later on it was doubted; because in light, due to the reverse pro-

cess of photosynthesis, respiration was masked. Sachs cleared up the mystery saying that both the processes go on independently in light. He also showed that in darkness, due to the combustion of the food materials, plants lost weight.

In typical cases of respiration oxygen is absorbed and carbon dioxide and water are liberated. The following simple formula may serve to explain the reaction.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

In such cases the *respiratory coefficient,* also known as respiratory quotient, which is the ratio of carbon dioxide to oxygen $\left(\frac{CO_2}{O_2}\right)$ is unity. This is generally written as R.Q.

Types of Respiration

In all cases normal respiration does not take place and thus the respiratory coefficient is either more or less than unity. The following are some of the various types of respiratory oxidations in plants.

1. Aerobic respiration:

A. Carbohydrates (complete oxidation):—

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 674$$

kg. cal. (Energy)

B. Fats (complete oxidation):—

$$C_{57}H_{104}O_6 + 80O_2 \longrightarrow 57CO_2 + 52H_2O + 8{,}064$$

kg. cal.

C. Specialised respiration in Crassulaceae:—

$$2C_6H_{12}O_6 + 3O_2 \rightarrow 3C_4H_6O_5 + 3H_2O + 386 \text{ kg. cal.}$$

malic acid

2. Anaerobic respiration:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + 21 \text{ kg. cal.}$$

3. Aerobic after anaerobiosis:

$$2C_2H_5OH + 6O_2 \longrightarrow 4CO_2 + 6H_2O + 653 \text{ kg. cal.}$$

The respiratory quotient in all these cases will be different. A simple experiment as shown in figure 27 can be set up to demonstrate this. Four test tubes are taken and inverted respectively in separate troughs of mercury. The one in which wheat is kept the

R.Q. $\frac{1}{1}$ $\frac{1}{0}$ $\frac{5}{8}\frac{7}{0}$ $\frac{0}{1}$

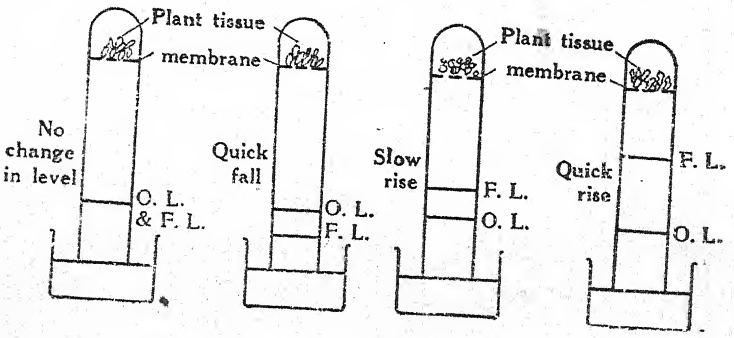


Fig. 27.—Diagram of experiment to show a simple way of demonstrating R.Q. in different plant tissues; F.L., final level; O.L., original level.

respiratory quotient is unity. While the other tubes show varying respiratory quotients.

The normal respiration is a complex of many reactions. Possibly the first reaction is anaerobic and

later on in the presence of oxygen the intermediate substances are completely oxidised to carbon dioxide and water.

As oxygen is required for aerobic respiration this gas has to enter into the leaves. Similarly the exhaled carbon dioxide has to pass out. Having once entered a particular tissue the gas has to reach quickly the myriads of cells composing the tissue. The intercellular spaces in plant tissues provide for the quick diffusion of gases within, while the stomata serve the purpose of the gate for gases within to communicate with the exterior. Stems are thick structures, so the gaseous diffusion is difficult. But in stems the activity of the cells as compared to the leaf is at a minimum. Here only the cambium is active and so the less efficient channels for gaseous diffusion than what is met with in leaves are sufficient. The intercellular spaces, connect with lenticels. There is always a well developed system of air spaces in the medullary rays by which oxygen diffuses to the cambium.

Roots also require aeration. If soil gets waterlogged the oxygen supply is cut down and the roots naturally are killed. Every water plant has a well

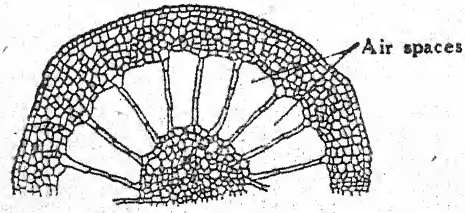


Fig. 28.—Cross sec. of **Myriophylum** stem showing air spaces.

developed system of air spaces with a complete system of tubular channels. In plants, growing normally in

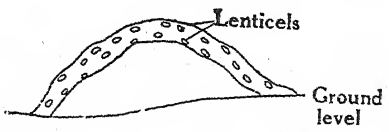


Fig. 29.—Diagram showing lenticels in aerial roots.

water-logged soil, aerial roots are developed which come above the surface of water. At the surface of such exposed roots lenticels are found through which gaseous diffusion can easily take place.

Stages in Respiration

As has been mentioned previously respiration is a vital phenomenon and so it is intimately connected with the condition of protoplasm, which is the physical basis of life. Thus, following the three stages of the protoplasmic condition, there are the three stages in respiration. These stages are as follows:—

1. *Embryonic stage.*—In early embryonic stage the respiration is low, as the very young protoplasm has not yet reached the high metabolic peak. But the respiration rapidly increases towards the late embryonic stage which is the stage of the plant's greatest activity.

2. *Mature stage.*—Here the respiration has practically reached the peak and the respiration rate steadily declines off with the age of the protoplasm. These two phases are graphically shown in figure 30.

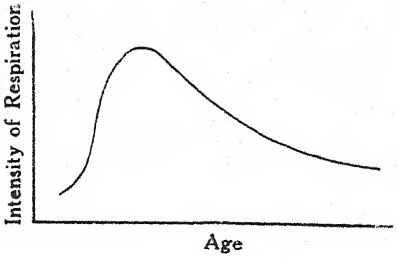


Fig. 30.—Graph illustrating respiration in embryonic and mature stages.

3. *The senescent stage.*—We now come to the senescent phase, which may be divided into early and late senescent phases. In the early senescent phase the respiration slowly rises to a peak. When the late sene-

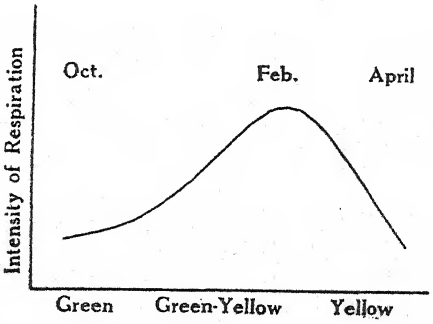


Fig. 31.—Graph illustrating respiration of apples as the fruits ripen and change colour from green to yellow, showing senescent rise and fall.

scence sets in, the respiration rate commences to climb down rather more rapidly. The work of Blackman and Parija on the apples shown graphically in figure 31 gives an idea of the respiratory drift of a ripening apple.

Respiration of a Starving Leaf

Blackman working with leaves of cherry laurel showed that when they are detached from the parent plant and allowed to respire in darkness the respiration rate which is high at the commencement starts climbing down rapidly with time. In a few days the respiration rate comes down to about a quarter of the initial respiration. But once having reached this level, instead of going still further down, it maintains this level for

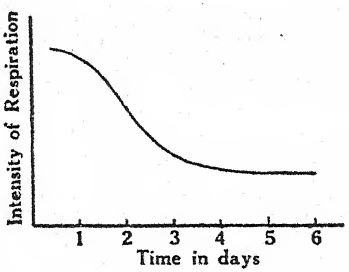


Fig. 32.—Graph representing respiration of detached leaves.

quite a number of days. The first phase of the respiration which ends at the point where the fall ceases is called the floating respiration by Blackman, and the low horizontal phase the protoplasmic respiration. See figure 32.

The exact cause of this fall in the respiration rate is yet a mystery. For it has been shown that even after a whole week in darkness the leaves show abundance of carbohydrates, though the percentage of the lower carbohydrates decrease. Ranjan has, however, shown that absence of light does cause a fall in the

respiration rate and when light is shown to the leaves at the protoplasmic level the respiration rate increases.

Conditioning Factors

As in photosynthesis respiration too is governed by external and internal factors. Amongst the foremost of these are (a) oxygen and (b) temperature, serving as external factors and (c) food supply and (d) enzymes, as the internal factors.

Oxygen.

Respiration being a slow oxidation process it is not surprising to find that the rate depends upon oxygen supply. It rises with high concentration of oxygen and drops gradually with diminishing partial pressures of this gas. When the supply of oxygen is cut down below a certain limit then partial anaerobic condition sets in.

Temperature.

Respiration being a physico-chemical phenomena it must obey the physico-chemical laws.

Formerly, however, it was thought that there was a certain minimum temperature below which respiration could not take place. Similarly, there was a maximum beyond which the cells were scorched and killed, while there was an optimum temperature at which the respiration rate kept the highest.

It has now been proved that Vant Hoff's Law of Q10 holds good for respiration also. This simply

means that for a 10° rise in temperature the respiration rate is doubled. This, however, holds good for low temperatures for at higher temperatures, due to the breakdown in the plant machinery, Vant Hoff's Law cannot be directly proved.

Food Supply.

Continuance of respiration must ultimately depend upon a steady rate of food supply. It is obvious, that substances must be oxidised in respiration. Palladin has shown that in normal cases carbohydrates are the substances that are oxidised. It is only when the carbohydrates are not available that proteins are attacked. Polysaccharides like starch are at first hydrolysed to the monosaccharides before they get oxidised.

Thus other factors being in excess the rate of respiration will depend upon the quantity of the monoses. The faster the hydrolysis of starch the higher will be the rate of respiration. All the monoses are not equally respired. It has been proved that the glucose sugar is the most easily respired, hence it is called the katabolic sugar.

The Mechanism of Respiration

The first step in the process of respiration is the hydrolysis of starch to sugar. In this reaction water chemically unites with starch, with the resultant formation of glucose. In the subsequent reaction glucose gets broken down into substances with half the number

of carbon atoms as are found in glucose. These intermediate substances then finally get oxidised.

Starch
Hydrolysis ↓
Sugar
Glycolysis ↓
Intermediate substances with half the number of carbon atoms of sugar.
Oxidation ↓
$CO_2 + H_2O$

As to how exactly the actual oxidation takes place in a plant is still obscure, but biochemical researches have thrown considerable light on this aspect of plant physiology.

Oxygen of the atmosphere undoubtedly plays an important part in aerobic respiration. It is well known that its activity differs according to its state. In molecular state it is not so active and it is in this state that it is present in the atmosphere. And in a dry state also it is very little active. The oxygen of water can be very active. So that if hydrogen from water is removed then atomic oxygen will be liberated and this is very active.

Palladium is one of the substances that can remove hydrogen from water and get itself reduced. The reaction is as follows:—

(1) $Pd + H_2O \longrightarrow PdH_2 + O$
(2) $PdH_2 + O_2 \longrightarrow Pd + H_2O_2$
(3) $H_2O_2 \longrightarrow H_2O + O$

Since the release of the free atomic oxygen in equation (1) involves the combination of hydrogen with some substance in the plant—in this case it is palladium —this process is called oxido reduction. Because simultaneously oxygen will oxidise the intermediate substances as given in the scheme above to carbon dioxide and water and on the other hand the hydrogen of water thus liberated will reduce a chemical substance within the plant with which it unites. Palladin has shown that there are respiratory pigments in plants which act like the palladium in the above equation.

Anaerobic Respiration

Unlike animals plants can live for a considerable period in the total absence of oxygen. Parija has shown that some apples can live for days together in the total absence of oxygen. At first it was thought that when plants respired in the absence of oxygen this gas was somehow or other supplied by the breakdown of the protoplasm. Hence the respiration in the absence of oxygen was called *intra-molecular respiration.* It has since been proved that in this type of respiration no oxygen is at all required, and that the process is very nearly akin to alcoholic fermentation. The name, therefore, of this type of respiration is changed to *anaerobic respiration.*

Alcoholic Fermentation

The origin of alcoholic fermentation is shrouded in the misty past. It has been known to mankind since

time immemorial. One finds it mentioned in the Vedas and the date of the Vedas may be anywhere between 3000—5000 B.C.

Our scientific knowledge of the process really starts from the date of the classical researches of Pasteur (1860) who succeeded in ascertaining correctly the biological nature of this process and in proving that fermentation is brought about by the activities of the yeast.

Yeasts belong to the genus *Saccharomyces*. These are single-celled organisms which rapidly multiply by budding. Unlike ordinary plant cells yeasts do not have a number of vacuoles in their cytoplasm, but contain a large vacuole in the nucleus itself Moreover, the cell-wall of yeast is composed of chitin, a substance peculiar to animals. In common with the rest of the fungi yeasts do not contain any chlorophyll and thus have to depend upon other sources of food supply. Yeasts are found growing naturally upon the surface of grapes. If grapes are bruised the sugary juice inside is at once attacked by yeasts and alcoholic fermentation sets in which can be expressed by the following simple equation.

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH + 21 \text{ Kg. Cal.}$$

It was Buchner who conclusively proved that fermentation may take place apart from the vital activities of the yeast cells. He ground up yeast with sand and then subjected it to a pressure of 300 to 400 atmospheres. The transparent juice which he thus obtained caused sugar to ferment. The enzyme of this

juice is called *zymase*. Recent studies have, however, shown that it is not a single enzyme, but a complex of several enzymes, which shows thereby that fermentation is not a simple process as depicted by the chemical equation given above. Moreover the products of fermentation have been found to be not only carbon dioxide and alcohol, but also appreciable quantities of fusel oil, glycerol and succinic acid.

Curiously enough the enzymes of the yeast are capable of attacking only certain types of sugars, such as d-glucose, d-fructose, d-mannose and d-galactose and these are also the sugars that are oxidised in respiration. The sugars of the l-series are not acted upon by plants.

In the presence of oxygen alcoholic fermentation decreases, though it never stops altogether. The reasons why all the alcohol is not oxidised in the presence of oxygen are twofold.

(1) Presence of poor oxidising enzymes in yeast.

(2) The rapid diffusion of alcohol out of the cells.

In a concentration of over sixteen per cent. of alcohol yeasts get killed.

Researches of Harden and Young and others have shown that fermentation takes place in a number of complex reactions employing inorganic phosphates as coenzymes. They conclude that the fermentation takes place in two stages. In the first phase a hexosediphosphate is formed. The second reaction consists of

hydrolysis by water of the hexosediphosphate to phosphate and hexose. The problem of hexosephosphatic esters in alcohol fermentation is, however, a complex one. Neuberg and his collaborates postulated the intermediate formation of methyl glyoxal. The oxidation and reduction of this substance was assumed to be brought about by a series of Cannizzaro reactions of the various aldehydes produced as intermediate products.

Boyland suggests the following sequence of reactions:—

(1) $3C_6H_{12}O_6 + 2R_2HPO_4 = 2C_6H_{11}O_5(PO_4R_2) + 2CH_3CO.CHO + 4H_2O.$

Methyl Glyoxal.

(2) $2C_6H_{11}O_5(PO_4R_2) + C_6H_{12}O_6 + 2R_2HPO_4 = 2C_6H_{10}O_4(PO_4R_2)_2 + 2CH_3COCHO + 4H_2O.$

Hexosedi phosphate

Phosphate involved in the above reactions and also a part of the hexose is liberated back in the following way.

(3) $2C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = 2R_2HPO_4 + C_6H_{12}O_6$

Part of the Methyl glyoxal formed in (1) and (2) undergoes simultaneous oxidation and reduction (first Cannizzaro reaction) producing glycerol and pyruvic acid.

(4) $CH_3COCHO + H_2 + H_2O = CH_2OH.CHOH.CH_2OH$ Glycerol.

CH_3COCHO (‖ O)

$+ CH_3CO.COOH.$

Pyruvic acid.

Pyruvic acid thus formed is acted upon by the enzyme carboxylase and split into acetaldehyde and carbon dioxide.

(5) $CH_3COCOOH = CH_3CHO + CO_2$

Methyl glyoxal and aceteldehyde now undergo a second Cannizzaro reaction under the influence of water to produce alcohol and more pyruvic acid.

(6) $CH_3COCHO + CH_3CHO + H_2O = CH_3COCOOH + C_2H_5OH,$

Fate of this pyruvic acid also is the same as given in reaction (5).

Thus the sequence of reactions continues, the main features being—

(i) no permanent loss or change of phosphate,

(ii) production of alcohol and carbondioxide, and

(iii) production of minute quantities of glycerol, pyruvic acid, acetaldehyde, etc. (ii) and (iii) taking place at the expense of hexose.

The above scheme has the virtue of combining the ideas of Harden and Young with those of Neuberg.

The poor oxidase system, the utility of the phosphate and the end products of alcoholic fermentation all point out that though there is a close similarity between anaerobic respiration and fermentation yet they are not identical. The table given below gives a comparative chart of the three types of oxidations.

Aerobic	Anaerobic	Alcoholic fermentation
Complex carbohydrates	Complex carbohydrates	Glucose or fructose
↓	↓	↓
Glucose	Glucose	3-carbon atom compounds
↓	↓	↓
3-carbon atom compounds	3-carbon compounds	Complex intermediate reactions
↓	↓	↓
$CO_2 + H_2O$	C_2H_5OH + CO_2 + some other substances	CO_2 + alcohol + glycerol + succinic acid

Bacterial Fermentations

The diverse activities of plants have brought out forcibly the fact that though the various chemical elements remain constant the compounds are for ever changing and in a state of circulation. Bacteria also play a very important part in keeping the matter in circulation.

The lactic acid fermentation is caused by *Bacillus lactici acidi,* which attacks the milk sugar (lactose) to produce lactic acid. Thus by the break down of a compound of higher energy content to a lower one energy is liberated which is utilized for the service of the organism.

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 4C_3H_6O_3$$

Similarly in the complete absence of oxygen *Clostridium butyricum* causes glucose to ferment into butyric acid;

$$C_6H_{12}O_6 \longrightarrow 2H_2 + 2CO_2 + C_4H_8O_2$$

while when lactic acid is fermented we again get butyric acid.

$$2C_3H_6O_3 \longrightarrow 2H_2 + 2CO_2 + C_4H_8O_2$$

The rancid smell in the butter, when it is kept for a number of days, is due to the activity of this bacterium.

Bacterial Respiration

In response to oxygen bacteria do not react the same way as the higher plants, otherwise the formation of the various chemical compounds in nature will not be possible.

The acetic acid bacteria (*Mycoderme acidi*) oxidises the alcohol to form acetic acid. We owe all our vinegar to the activity of this bacterium.

$$CH_3CH_2OH + O_2 \longrightarrow CH_3COOH + H_2O$$

Similarly the sorbose bacteria attack sorbite—an alcohol—to yield sorbose according to the following equation.

$$2C_6H_{14}O_6 + O_2 \longrightarrow 2C_6H_{12}O_6 + 2H_2O$$
Sorbite Sorbose

Practical Experiments

Exchange of Gases.

1. *Experiment to show the production of CO_2 in the normal respiration of a plant.*—The apparatus is fitted as demonstrated in the experiment. A green potted plant is placed over a glass plate and inside a bell jar which is made air tight by smearing vaseline. The bell jar is covered with a black cloth. The U tube is

filled with soda lime. The other two bottles on either side of the bell jar contain barium hydroxide solution. Air is sucked in through the apparatus by an aspirator. The air after passing through the soda lime is made free of CO_2 which becomes clear by the fact that barium hydroxide in the bottle just after the U tube does not turn turbid. The barium hydroxide in the bottle on the other side of the bell jar turns turbid showing that CO_2 has been given out by the plant during respiration.

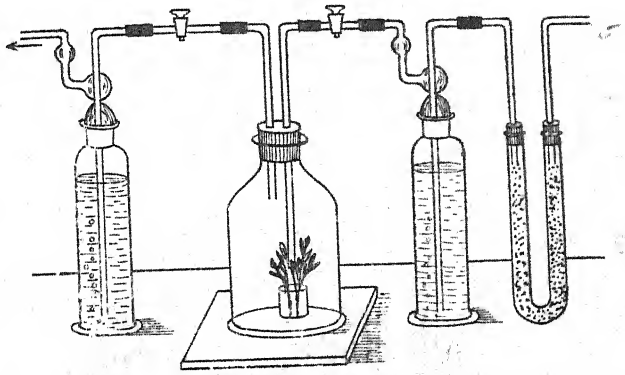


Fig. 33.—Diagram of apparatus to show the production of CO_2 by a plant (Black cloth covering the plant chamber is not shown).

2. *Experiment to show the production of CO_2 in the respiration of roots.*—The above experiment is repeated on roots and the same results are obtained.

3. *Absorption of CO_2 by potash.*—In a bottle some germinating seeds are put. A test-tube filled with strong KOH solution is introduced into the bottle. To a side opening of the bottle is attached a glass tube whose end dips in a beaker containing mercury. As the CO_2 produced by respiration is absorbed by the KOH, the mercury in the beaker is sucked up into the connecting glass tube.

4. *Experiment to show the absorption of oxygen in respiration and to determine the respiratory coefficient RQ.* (CO_2 /O_2).—The apparatus is fitted as shown in figure 34. The glass dish inside the bell-jar

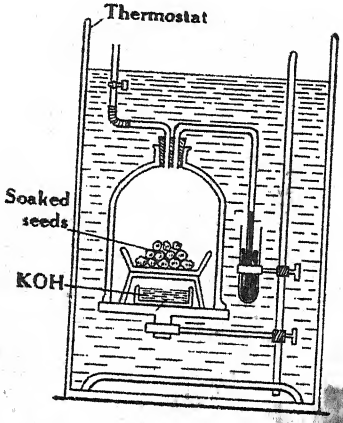


Fig. 34.—Diagram of apparatus to investigate R.Q.

contains strong KOH solution. The outer glass case in which the apparatus is put contains water to keep the temperature constant. During respiration of the germinating seeds oxygen is absorbed and CO_2 is given out. The amount of oxygen taken in by seeds is shown by the rise of the level of mercury; and by weighing the KOH solution we can know the amount of CO_2 evolved. From these data the R.Q. can be calculated.

5. *To determine the respiratory coefficient by means of the respirometer.*—Some germinating wheat seeds are placed in the bulb of the respirometer (Fig. 35). As the R.Q. is unity in this case there is no rise in the level of mercury.

6. *Experiment to show the production of CO_2 in normal aerobic respiration.*—Germinating seeds are placed in two tubes the ends of which dip in beakers

of water. In one of the tubes a small test tube containing strong KOH solution is introduced. This tube shows a rise in the level of water but the other tube does not. Explain.

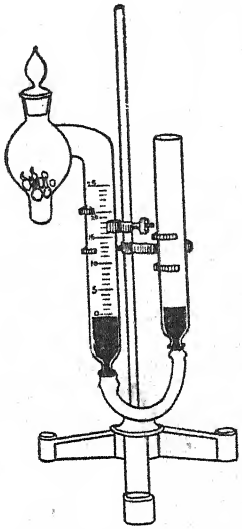


Fig. 35.—A respirometer.

7. *Production of CO_2 in anaerobic respiration.*—A few germinating seeds are introduced in an inverted tube completely filled with mercury. After a time the level of the mercury is pushed down by the CO_2 evolved during anaerobic respiration. If a little potassium hydroxide solution is introduced into the tube through the lower end, the CO_2 is absorbed and mercury again fills the entire tube.

8. *Experiment to compare aerobic and anaerobic respiration.*—Two flasks are taken and some germinating seeds are placed in each. Inside one flask a test-tube containing pyrogallic acid is introduced and in the

other a tube filled with red alkaline phenolphthalein, is placed. Glass tubes passing through the cork dips in each of these. After a time it is seen that the phenolphthalein loses colour due to absorption of the CO_2. In the other flask the pyrogallic acid will deprive the air inside of all oxygen. Anaerobic respiration will set in and the production of CO_2 will cause the level of the pyrogallic acid to rise in the tube.

9. *Experiment to show the production of CO_2 in fermentation.*—To a solution of grape sugar in a flask some yeast is added. CO_2 which is evolved during fermentation turns the baryta water, kept in another flask connected with the former by a tube, milky.

Respiration in Various Tissues.

1. Take a little baryta water in a test tube. Put some cotton inside the tube and push it in so that it lies just above the level of the baryta water but does not touch the baryta water. Place some green leaves on the cotton and keep the whole thing in sunlight for about 15 minutes. Observe that there is no turbidity in baryta. Now place the test tube in the dark for the same period. Note that baryta has become turbid due to the evolution of CO_2 and the consequent formation of barium carbonate.

2. Repeat the above experiment separately (*a*) with germinating seeds, (*b*) with cactus, and (*c*) with *Bryophyllum*. Note the results.

3. *Experiments on respiratory co-efficient in plants.* (See Fig. 27, page 90). The experiments are fitted to demonstrate as follows:—

(*a*) *Germinating wheat seeds*—No change in the mercury level. Showing that the RQ is unity.

(*b*) *Fatty seeds.*—The mercury rises. Showing that more O_2 is taken in and less CO_2 is evolved, *i.e.*, R.Q. is less than unity.

(*c*) *Cactus.*—Great rise in mercury. Showing that O_2 is taken in but no CO_2 is given out. During oxidation acids are formed.

4. Cut T. S. of a leaf and a stem and study the intercellular spaces in leaf and in the medullary rays of the stem. Note the efficient system of gaseous interchange in both the stem and the leaf.

5. Study the aeration system in the aerial root of *Kandelia* (mangrove). Note the lenticels and the air-spaces, which are connected with them. The cortex is quite thick.

FACTORS AFFECTING RESPIRATION.

1. *Effect of temperature on respiration.*—In a glass chamber germinating seeds are put. Air freed from CO_2 is drawn through it into a bottle containing baryta water. After a certain time the turbidity of baryta is noted. The chamber is placed in a beaker containing warm water and the turbid baryta is replaced by an equal quantity of fresh baryta. The turbidity produced now is found to be much greater than before, showing an increase in respiration.

2. *Effect of O_2 on respiration.*—In two glass chambers germinating seeds are put. Through one of these air free from CO_2 and O_2 is drawn by passing it through soda lime and pyrogallic acid. Through the other air freed only from CO_2 is passed. It is seen that the baryta in the latter is much more turbid, showing that CO_2 evolution is much greater in the presence of oxygen.

3. *Effect of food-supply on respiration.*—The respiration, as seen by the turbidity produced in baryta, of

a leaf which has been starved by keeping it in darkness for 24 hours, is compared with that of a similar leaf kept in light. It is seen that the amount of respiration in the latter case is much greater than in the former showing that an increased foodsupply results in greater respiration.

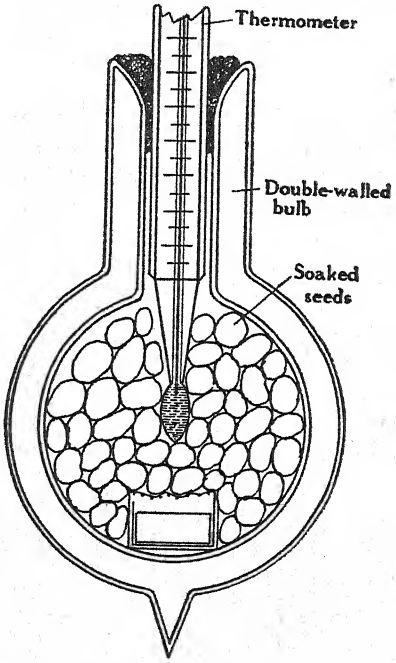


Fig. 36.—A double-walled vacuum bulb to show rise in temp. during respiration.

4. *Experiment to show the rise of temperature during respiration.*—Two double-walled vacuum bulbs are taken. In one of them some germinating seeds are put and the other is kept empty. In each of these a thermometer is inserted. After a time the temperature inside the bulb with germinating seeds is seen to rise.

CHAPTER VI

THE TRANSPIRATION STREAM

The Problems of Water Supply

It has been aptly said that " All living matter lives in water ". If a plant is deprived of water it will die. As a matter of fact protoplasm is mostly water. In extreme cases such as cucumber or watermelon 96% of the weight of the plant is just water. If this water from the protoplasm is removed, growth and other metabolic functions decrease and ultimately stop. The seed which contains relatively little water remains dormant and bursts forth into activity only when water is supplied.

The problem of the water supply to the fruits, the seeds, the leaves, and the growing tips is an acute one, in a land plant. To a free swimming plankton the water supply is not a distressing problem. It can easily absorb the surrounding water by osmosis. But a tall tree has to carry its water from the soil or the subterranean regions of the soil through special channels to remote parts of the plant.

Before going further into the details of water movements inside the plant a knowledge of the soil is essential.

Soil and their Relation to Plant Life

The common soil on which plants grow is a complex substance, produced by the wear and tear of

rocks. The agencies responsible for the breakdown of the mighty rocks are rain, wind, sunshine, and frost.

Rain water enters the crags and in winter gets frozen and thus expands. By the enormous force of expansion the rocks crack and the rain finally washes the cracked rocks down. During their downward course, they get rubbed and broken till ultimately they assume the form of silt.

Plants like lichens also are responsible for the breakdown of these rocks. Lichens can grow on bare rocks and as they secrete certain organic acids the rocks get corroded. The rain water now enters these corrosions and on freezing complete the work of destruction.

The various stages of the formation of the soil can be seen in a quarry. An example can be met with in a natural section of a quarry where stones are being broken for metalling the roads. The section shows the transition of the fine surface soil to the lower coarser soil. Below this there are layers of pebbles forming the lower subsoil and finally one finds the massive unbroken rocks below.

Most rocks of the world are of the *sedimentary* kind. This includes all groups of rocks which have been formed by the accumulation of sediments generally deposited under the sea or lake. These sediments, being composed of mud and clay, are washed out far into the sea being lighter than sand. These are then deposited layer by layer one above the other, and eventually when the sea recedes, they form dry land.

The various layers are called *strata*. Nearly all the rocks of the sedimentary type show strata.

Sedimentary rocks or soils contain calcareous matter also called lime stone particles. These are very useful as they serve to neutralize the organic acids produced by the decay of the vegetables. When once the soil is formed nature tills it. Here the earthworms play a very important part. On an average there are about 50,000 individuals on an acre and they continually turn out the soil slowly, invisibly, but efficiently.

Soil Structure.

The chief constituents of a soil are:—

(1) Quartz and mica—in the form of sand and finer silt.

(2) Clay—the finely divided colloidal silicates of aluminium.

(3) Humus—the organic colloidal matter.

(4) Calcium carbonate—derived from chalk and lime stone.

A mixture of these goes to form soils. The latter are of three kinds, *viz.*, (*a*) loamy, (*b*) sandy, and (*c*) clayey.

The differences in the three are mainly due to the differences in the proportions of the various constituents that go to form the soil.

A good soil is usually known as *loam* and it contains:—

Sand	...	...	60 %
Clay	...	...	25 %
Humus	...	...	7·5 %
Calcareous matter		...	7·5 %
			100

The differences between sand and clay is also in the size of the particles. Clay particles are very much smaller. Each, however, has its own usefulness. Sand grains help to keep sufficient spaces for water and air between the particles, of the soil. But too much sand is also bad, as for instance, in the deserts where the soil becomes too porous and thus is dry. Clay particles exert a moderating influence. But this too in excess causes the soil to be heavy, cold, badly drained, and not sufficiently aerated. Clay absorbs potash, lime, etc. when these are added to the soil, and thus they are saved from being washed away.

Colour of the Soils.—Soils are generally dark coloured due to the presence of decaying plant material by the action of bacteria. These decaying mass of vegetation is called *humus.* This supplies most of the nitrogenous matter. The *subsoil* is generally light coloured. The depth or thickness of the soil covering the surface of the globe varies from a few inches to a few feet. In India, generally the thickness of the soil does not exceed 6″ .

Physical Aspect of Soils.—The crumby property of a loamy soil is due to the fact that it has got a definite

structure and it is not merely a simple mixture of various substances compounded together. If some soil is shaken up with water and then dried, it looses its crumby property when it is again moistened, showing that it has lost its definite structure. In agriculture this fact is of utmost importance for, when too wet a soil is worked it becomes puddled loosing its crumby nature and then it becomes difficult to bring back the original condition.

The most important constituent of the soil is calcium carbonate. It tends to have a mild alkaline reaction. Acid soils are not good for vegetation, and so are highly alkaline soils. Incidently, barren soils in India are generally alkaline.

Soil Moisture.

Soil moisture can be resolved into three subheads:—

(1) *Gravitational water.*—After a heavy shower the excess of water sinks below and is drained away.

(2) *Capillary moisture.*—Some water is held by the soil particles by capillary action.

(3) *Hygroscopic moisture.*—This amount is equal to the amount of moisture taken up by a dry soil in wet atmosphere.

Wilting in Plants.

The amount of water retained in different soils show that clayey soils hold the most moisture, then

comes loamy and last of all sandy soils. Briggs and Shantz have carried out extensive researches in this line. They have shown that though clayey soils contain more moisture than sandy soils, yet the plant is not able to remove most of the water held by the clayey soil. Their data show that finer the soil particles the greater is the amount of water remaining in the soil when the plant dies from the lack of moisture supply.

When the soil moisture is so low that the rate of absorption of water from soil is less than the rate of transpiration the leaves gradually become flaccid. Such a condition in general is known as wilting in plants. Temporary wilting is that when the flaccid leaves regain their turgidity in a more humid atmosphere. If, however, the normal turgidity will not be regained even in a saturated atmosphere without the addition of water to the soil then that condition is known as permanent wilting. Even after permanent wilting plants continue to absorb moisture from soil, but the process is very slow and indicates only a mechanical transfer from soil to air. Moisture content of the soil in terms of percentage of the dry weight at the time of permanent wilting is termed by Briggs and Shantz (1912) as *wilting coefficient.*

Wilting coefficient can be determined by the application of "direct method." In this method the plant is allowed to grow in a sealed container and at the stage of permanent wilting, a sample of the soil is taken from the same pot and analysed for the percentage of

moisture on the dry weight basis. The value so obtained is known as the " observed wilting coefficient."

'Direct method' of determination of wilting coefficient is possible with small plants which can be grown in small pots. With bigger plants grown either in big pots or in the field, neither the soil condition round the plant is uniform nor, the wilting is uniform and sharp. in different parts of the plant. In such cases various indirect methods are used for finding the so called " calculated wilting coefficient " values.

One such indirect method is based upon the moisture equivalent of the soil which means the percentage of water that soil can retain in opposition to a centrifugal force 1000 times that of gravity. On the assumption that wilting coefficient is practically the same for a given soil for any plant under any climatic condition, Briggs and McLane (1907), with the help of ' direct method ' of determination of wilting coefficient reached the conclusion that with any soil,

$$\frac{\text{Moisture Equivalent}}{\text{Wilting Coefficient}} = 1{\cdot}84\ (1 \pm 0{\cdot}007).$$

Same equation expressed in the following way may be used to determine the wilting coefficient.

$$\text{Wilting Coefficient} = \frac{\text{Moisture equivalent}}{1{\cdot}84\ (1 \pm 0{\cdot}007).}$$

The Water Absorbing System

The root system is mainly responsible for the absorption of water. These are of various types according to the nature of plants. Erroneous idea may

be formed regarding the total length of a root system by uprooting a plant and examining its roots. The small rootlets are in so intimate a contact with the soil particles, that they invariably get broken. It is when plants are grown under special conditions that the root system can be properly examined. Nobbe has shown that the aggregate length of the roots of a full grown wheat plant may be as much as 600 yards. That of a full grown vine may be about 15 miles.

Roots and rootlets, however, hold a secondary position in respect to their relation vis-a-vis the plant and the soil water. For, the root hairs form the primary link. The latter are developed a little way behind the root tip. The region of the rootlets where the root hairs develop ceases to grow otherwise the delicate root hairs may get torn.

The elongation of the rootlets into the soil takes place very near the tip for obvious reasons. If one tries to push in clay a fine wire from top it will get bent, but if it is caught hold of nearer the tip and then pushed no bending will take place.

Root hairs are short-lived: the average life being from a few days to a month. When the soil is heavily watered the production of root hairs is curtailed to a certain extent. In liquid cultures the root hairs keep straight but when grown in the soil they get bent (Figure 37) and contorted around soil particles. Thus they come into intimate contact with each soil particle. The root hair consists of (1) a cell-wall, (2) an external plasma membrane or ectoplast, (3) a general cytoplasm,

and (4) an internal plasma membrane or the tonoplast. The cavity encircled by the tonoplast is filled with cell sap containing sugars, acids and minerals.

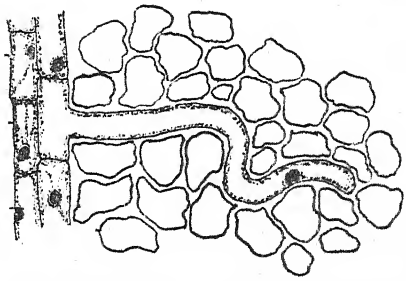


Fig. 37.—A young root hair pushing through soil particles.

An artificial semipermeable membrane like the plant cell was made by Pfeffer. He took a porous clay cell within which he poured a solution of copper sulphate. He then dipped this cell in a solution of potassium ferrocyanide. While the copper sulphate

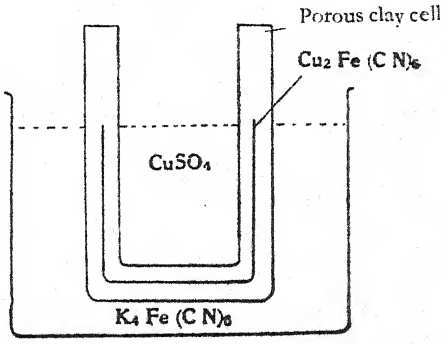


Fig. 38.—Diagram illustrating Pfeffer's artificial membrane.

oozed out of the porous cell, the potassium ferrocyanide penetrated inside and at the junction of the two within

the porous cell-wall semipermeable membrane of copper ferrocyanide was deposited.

The membrane thus deposited can withstand great pressure. The porous cell is analogous to the cell-wall and the tensile strength is like that of steel.

Function of the Protoplast, Cell-Wall and Vacuole System

The vacuole system is the fundamental system found in the vast majority of adult plant cells and the entire metabolic machinery is based upon this system. It is due to this that the plant cells possess turgidity.

The turgid condition of a normal healthy plant cell is due to the osmotic property of the cell sap which causes the suction of water inside the vacuole whereby the protoplasm gets distended causing it to press against the inelastic cell-wall, much in the same way as an inflated football bladder presses against the outer leather. If a manometer were to be inserted in a cell, the tension would be released and some of the liquid from the vacuole pressed out. To put back that liquid the hydrostatic pressure required may be as much as 35 atmospheres.

Vant Hoff was led by thermodynamical considerations to the view that the pressure developed by substances in solution is identical with the gas laws; thus the solute behaves as if it were in the dispersed molecular condition of a gas and the solvent was absent. At a constant temperature the osmotic pressure, then, varies as the density or the concentration of the solute.

The *osmotic pressure* can be defined as a pull that the cell sap is capable of exerting upon pure water if it were separated from it by a perfectly semipermeable membrane. The osmotic pressure of a cell is generally expressed in terms of atmospheric pressure.

A semipermeable membrane is defined as one that will allow only the solvent to pass and not the solute; goats bladder and collodion are examples. An absolutely perfect semipermeable membrane is not found in nature. It always does allow some amount of the solute to pass through it.

If one gram molecule of glucose is dissolved in a litre of water it exerts a pressure of 22·4 atmospheres.

Thus:—18	%	glucose	exerts	22·4	atm.	pressure.
8	%	,,	,,	10	,,	,,
6·9	%	,,	,,	8·6	,,	,,
6·0	%	,,	,,	7·5	,,	,,
5·36	%	,,	,,	6·7	,,	,,
4·8	%	,,	,,	6·0	,,	,,

But 3% calcium nitrate exerts 7·5 atm. which is equal to the pressure exerted by 6% glucose. This is because the molecules of calcium nitrate get dissociated into ions, while in the former case the molecules do not break up.

Assuming then that a protoplasmic membrane is impermeable to the solutes, if we put a plant cell in water, no diffusion of solutes into the cell across the membrane could occur. Since the concentration of

solutes in the interior of the cell is higher than that of water, the latter will diffuse inwards into the vacuole by the process of osmosis. The vacuole will grow in size and, exerting a hydrostatic pressure, will push the cytoplasmic layer centrifugally towards the cell wall. This centrifugal pressure is an equivalent of the osmotic pressure and in its relation against the cell wall is called the turgor pressure. The cell wall now commences, in an increasing amount, to exert an inwardly directed pressure. When the limit of extensibility of the cell wall has been reached its inward pressure or centripetally directed pressure becomes equal to the turgor pressure and no more water will enter. This inwardly directed pressure is called the wall pressure. When a cell is fully turgid, the osmotic pressure, turgor pressure and wall pressure are at their maximum and equal to each other.

We thus see that when osmotic pressure equals wall pressure then no water can enter. Thus even though the value of osmotic pressure may be very high, the water may not enter a cell. The rate of flow of water consequently depends upon the *suction pressure* of a cell which is the difference in osmotic pressure of internal and external solutions less the wall pressure. Thus if osmotic pressure equals wall pressure the suction becomes zero. In this condition no water can enter however high the osmotic pressure may be. According to Brown the suction pressure is a force per unit area with which water would begin to enter the cell, if the cell were immersed in pure water.

Lateral Transfer of Water in Roots

Having considered the osmotic mechanism by which the soil water enters the root hairs, we may now consider the lateral transfer of water.

Working with *Vicia faba*, Ursprung and Blum found the suction pressure to rise from 1·1 atmospheres in the piliferous layer to 4·1 atmospheres in the cells just outside the endodermis. With the gradual increase of the suction pressure one can easily explain the transfer of water across the cortex towards the endodermis. But, then, the authors also found that there is a marked drop in the osmotic pressure within the endodermal cylinder. Therefore, there comes the difficulty of explaining as to how the water gets transferred from the cortex, a region of higher osmotic pressure to the endodermis, a region of lower osmotic pressure.

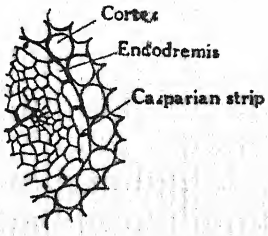


Fig. 39.—Cross section of a root showing Casparian strips on the radial walls of the endodermis.

Priestley has very ingeniously explained it on the grounds that the endodermal cells are radially thickened and contain the Casparian strips. These strips are impregnated with cutinised material thus effectively checking any flow of water radially through the walls

which may and does take place in the cortical region in cases of lateral water supply to the roots. In the endodermis the water therefore has to flow through the cell protoplasm, as follows. When the cells of the cortex are fully turgid the suction pressure becomes zero and as the endodermal cells, as also the cells within, are not so fully turgid, due to the constant removal of water from the xylem vessels, the suction pressure is considerably more than that outside the endodermis. Water is consequently drawn in.

Once the water has reached the endodermis its flow towards the tracheae can take place either along the wall of the cells or from cell to cell through the increased suction pressure gradient as in the case of flow through the cortex.

The next problem and which is more difficult to answer is to explain the mechanism of the entrance of water into the tracheae. For this purpose various theories have been suggested, which, however, are all hypothetical and are not supported by experimental evidence.

The theory of Lepeschkin is based on his observations on the guttation from the surface of the sporangiophores of *Pilobolus*.

It is well known that this fungus is coenocytic. When one end of the hyphae is dipped in water it is found that it exudes out from the various parts of the sporangiophores. He also showed by plasmolytic experiments that the sporangiophores are more permeable than the lower parts. Taking this as an analogy

Lepeschkin explains that the cell next to the xylem vessel has differential permeability at different sides; so that the side towards the xylem is more permeable than the side away from it. Thus when the water is sucked from a neighbouring cell into the cell next to the xylem, as the side towards the xylem is more permeable, the cell as a whole is unable to retain that water which consequently gets injected into the xylem vessel.

On the other hand, this unidirectional flow of water is explained by Priestley on the grounds that the cell next to the xylem vessel when fully turgid is unable to suck any more water and thus the suction pressure becomes zero. The dead tracheal elements, on the contrary, as a result of the excreting activity of the living cells adjoining them, are not subjected to the pressure of the impermeable protoplasm. Here, then, the suction pressure is equal to the whole of the osmotic pressure. It must, therefore, withdraw water from the neighbouring turgid cells as shown diagramatically from Priestley's figure.

One end of A, containing any strong solution, is immersed in a vessel of water B; while its other end adjoins the semipermeable membrane of C, whose upper end is open. C contains a solution of certain concentration. Now A withdraws water from B till it is fully extended and the suction pressure then becomes zero. In C osmotic pressure is equal to suction pressure because one end being open no turgor pressure develops. Water will, therefore, be drawn into C, indepen-

dently of the magnitude of osmotic pressure in A, *i.e.*, even if the osmotic pressure in C is less than that in A, the suction pressure in C being greater water will flow into C.

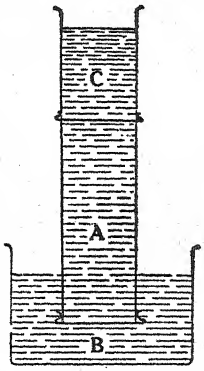


Fig. 40.—Diagram illustrating the transference of water into the dead xylem cells. (Explanation in the text).

Priestley thus makes the startling though quite logical suggestion that it is the dead vessel and not the living cells that draws water from the soil.

Root Pressure

The above theories of Lepeschkin and also of Priestley may serve to explain the mechanism of *root pressure*. This may be defined as a pressure set up in the fibrovascular bundle of the stem and root due to the water which is being froced in them. This pressure is noticed in the bleeding of cut vines, as also in "Neem" trees (*Melia azadirachta*) when occasionally the trunk by itself splits and the sap oozes out for days

together. Though undoubtedly Priestley's explanations of the mechanism of the entrance of water into the vessels does explain to a certain extent the mechanism of root pressure, it cannot be wholly explained by means of osmotic and imbibitional forces alone. It has been shown that changes of oxygen concentration and also temperature affect root pressure which thus points out the vital relations of the cell involving energy transference. Under conditions of little or no transpiration and an abundant water supply, a cut stem of a potted plant may show positive root pressure while under adverse conditions of water supply and high transpiration *negative root pressure* may be found.

Ascent of Sap

Various theories have been suggested to explain the raising of water from the roots to the leaves. The theories may be divided into two main classes: (1) the *physical* and (2) the *vital*.

The authors of the physical theories contend that the nature of the major forces concerned in the elevation of water is purely physical and obey the physico-chemical laws, though the living cells of the plant may also play a minor part as in the case of lateral transference of water which is an osmotic phenomenon intimately connected with the living protoplasm. The upholders of the vital theories, on the other hand, assume that the forces concerned are of a vital nature intimately connected with the living cells of the root,

stem and leaves and that the dead tracheal elements have next to no part.

PHYSICAL THEORIES.

Boehm's theory.—Boehm's views (1809) were that partly the capillary forces of the trachea and partly the atmospheric pressure were responsible for the ascent of sap.

Jamin's chain.—Jamin suggested that inside the stem there are alternate layers of water and air and by the expansion of air as also by lateral stresses like bending etc., the air moves up carrying the water column above it. By means of casts of plaster of Paris he demonstrated that even when there was a suction of less than one atmosphere from the top, water was raised to over 12 metres. The experiments were, however, defective as plaster of Paris has a property of absorbing water.

Imbibition Theory.—Sachs (1878) suggested that water moved up entirely in the walls of the xylem elements by the process of imbibition and that the lumen played no part. This view was, however, exploded by the work of Dixon and others who showed that when lumen of the vessels were blocked by gelatin, ice, water vapour, etc. severe wilting occurred.

Cohesion Theory.—This is at present the most accepted theory and was propounded by Dixon and Joly in 1894. This is based on two essential facts.

(*a*) The cohesive quality of water i.e., its quality of resisting tensile stress.

(*b*) The imbibitional force of the evaporating cell-walls of the leaf.

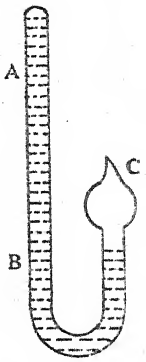


Fig. 41.—Diagram of Dixon's tube to show cohesive force of water.

To prove the cohesive quality of water Dixon took a bent glass tube sealed at one end and then thoroughly cleaned it and wetted the inner surface. He then filled it up with boiled distilled water up to two-third capacity of the tube and then sealed it. When the tube was gently brought to the horizontal position the water filled up the long arm of the tube depicted as A B in the figure. Now when the tube was gently lifted up to a vertical position the water kept adhering to the top and did not fall off to C. This was due to the surface molecule of water sticking to the side of the tube A B and also due to the cohesive quality of the water molecules. If one were now to tap the bent arm at B then there may occur a break in some part of the water column and the water will drop down with a metallic clang and fill up the space at C.

Dixon next took a similar tube and filled it up completely with dust free water only leaving just a little space for an air bubble and then sealed off the mouth. On carefully heating the tube the water expanded covering the entire internal volume of the tube and completely dissolving the air bubble. Then it was allowed to cool; but the water due to its adhesion to the glass surface resisted the strain of contraction. Only when the strain exceeded 7·5 atmospheres as calculated by Dixon, that the continuity was broken and the water occupied its original volume leaving the space free for the air bubble. Similar experiments showed that plant sap withstood a tension of 45 atmospheres before it was ruptured. Rupture occurred in unboiled sap at a tension of about 207 atmospheres. This according to Dixon may be due to the colloids present in the cell sap.

The cohesion theory assumes that the water is in the form of unbroken columns in the conducting tracts of the xylem which are continuous with each other both vertically and laterally through the cell-walls. The terminal endings of this water column are, on the one hand, the menisci of the water in the extremely minute cavities of the epidermal cell-walls of the roots and, on the other, those in the hypodermal cells of the leaves.

But there are cases where air bubbles may enter a xylem vessel and thus break the continuity of the water column. In such cases also, Dixon has shown that the continuity may not get com-

pletely broken as suggested by figure 42. Even the tracheae have cross walls at certain distances and

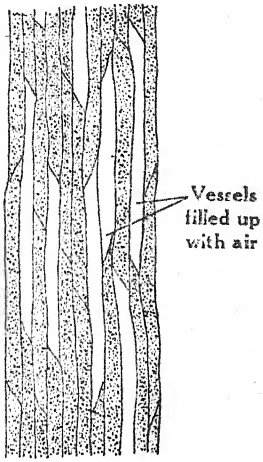


Fig. 42.—Diagram illustrating continuity of water in xylem vessels.

if in one trachea an air bubble gets introduced the continuity of the water column may be kept up.

Regarding the imbibitional force of the evaporating cell-walls of the leaf, it has been shown to be enormous. Dixon calculated that it would require a pressure of 100 atmospheres to break the continuity of the water column in the mesophyll cells of the leaf. The suction power of the vacuoles of the leaf cells, which is from 10 to 20 atmospheres, is ample to move the column of water in stems at an adequate rate to replace the water evaporated from the leaves. In the process of transpiration water particles are first set free into

the intercellular spaces of the leaves from the cell-walls. The surface tension of water in the cell-walls then increases resulting in the witdhrawal of water from the protoplasm into the cell-wall. The water from the vacuole, in its turn, is drawn into the protoplasm. Thus by the loss of water from the vacuoles of the cells the osmotic pressure rises which causes the flow of water into these cells from neighbouring cells. The disturbance in the dynamic equilibrium is transmitted eventually to the water in the xylem elements of the leaf and the pull is carried down through the xylem vessels of the stem and the root, ending finally in the root hairs.

Vital Theories of the Ascent of Sap.

Godlewski (1884) assumed a rhythmic change in the permeability of the medullary ray cells of the xylem, so that the water was taken up first from the xylem elements situated just below it and then released into the xylem elements above. In short the medullary ray cells acted as a pump and the xylem as the conduits for the water to flow.

Janse (1887) supported Godlewski and showed that if the lower portion of a branch was killed, the leaves above were, within a few days, affected. These theories, however, had no experimental backing.

Bose and Molisch were the recent supporters of the vitalistic view.

Bose (1923) believed that there was a layer of cells in the stem in a state of active pulsations which

caused the rise of water. He investigated this by his electric probe. One terminal of a galvanometer was connected with the probe and the other at some point in the plant as shown in Figure 43. The insulated fine probe was inserted into the stem very gradually.

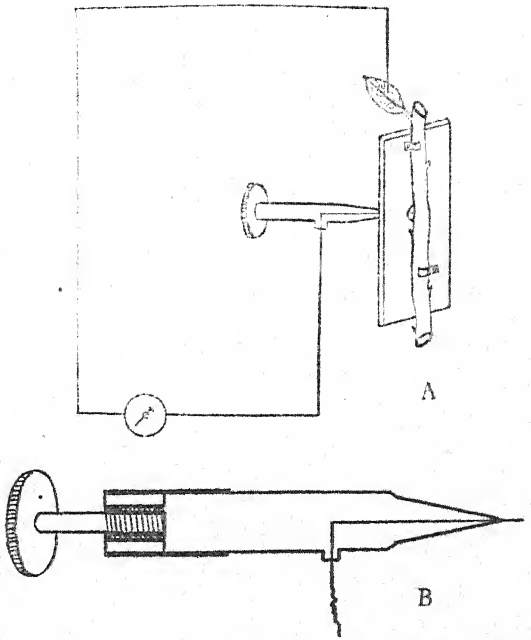


Fig. 43.—Diagram illustrating Bose's experiment with electric probe: A, the general arrangement of the apparatus; and B, the details of the probe (after Bose).

As the needle penetrated into the cells due to local irritations slight momentary oscillations were shown by the galvanometer, which after a time subsided. When the needle had reached one particular region

greatly be increased with drugs that cause increased cardiac activity in the animals.

Dixon and his co-workers, however, have adversely criticized Bose and have been unable to verify his results. Shull, MacDougal and others have also criticized Bose on the ground that he has not been able to correlate the relationship of the pulsatory activity to the ascent of sap. It has been estimated that the sap must flow through 200 to 400 pulsating cells per second to account for the normal rate of upward flow of the sap. This rate, naturally, is inconceivable from the rate of pulsations as found by Bose.

A schematic representation of the course of water in transpiration is given on page 135.

Factors Governing the Rate of Transpiration

The following are some of the main factors that influence the rate of transpiration:—

1. Leaf structure
2. Light
3. Wind
4. Temperature
5. Humidity
6. Water content of the soil.

Leaf Structure.

The leaves of most angiosperms are composed of thin walled parenchymatous tissue, through which finely divided vascular bundles ramify. The entire leaf

is covered by a single layer of epidermal cells with their outer walls somewhat thickened. The epidermis is perforated by minute pores called stomata (singular stoma). These stomata open into cavities within the leaf. These cavities in their turn are connected with the intercellular spaces which are invariably found in leaves due to the loosely packed nature of the parenchymatous cells. As has been said before, the water from the vascular bundles is drawn into the parenchymatous cells from where it enters into the intercellular spaces as water vapour and then by diffusion escapes out into the atmosphere through stomatal pores. Thus the structure of the leaf, the position of the stomata, and the factors that influence the stomatal opening will all affect the rate of transpiration.

Modifications of the leaf structure and the positions of the stomata on the leaf will be dealt with later. Here we have to note how a mesophytic plant regulates its transpiration rate by the movement of the stomata.

The Stomatal Apparatus.—The stomata are minute openings through the epidermis of plants. Except on the roots they may be found anywhere. They are, however, found in abundance on the leaves. In the first stage of its formation a young ellipsoidal epidermal cell divides into two daughter cells. In the next stage the dissolution of the middle lamella of the common wall separating the two daughter cells takes place resulting in an intercellular passage into the

interior. The two daughter cells are now called the guard cells.

By the variations in the turgor pressure in the guard cells the size of the stomatal opening is regulated. When the osmotic pressure in the guard cells increases water is drawn in from the surrounding cells causing the guard cells to distend. The thickness of the cell-wall of the guard cells away from the opening is less than what it is towards the side of the opening as shown in Fig. 45. When the guard cells

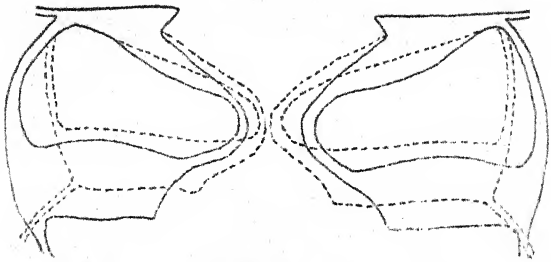


Fig. 45.—Cross section of a stoma and guard cells (diagrammatic) illustrating opening and closure of the aperture by the guard cells.

become turgid the thinner side, which is the side away from the pore, swells out as shown in the figure and consequently draws the thicker side, which is the side towards the pore, towards it. By so doing the pore is naturally widened.

Unlike the other epidermal cells of the leaf that are devoid of chloroplast, the guard cells contain green plastids. These, however, are not chloroplasts. Unlike the true chloroplasts which cannot be formed in the absence of light, the green plastids of the guard cells can be formed. There is also some doubt as to

whether they can manufacture carbohydrates. The starch present in the guard cells appears to have been translocated there from the mesophyll cells.

Factors Responsible for Stomatal Regulation: (*a*) *Light.*—The action of light upon stomata are somewhat peculiar. As has been said before, the green pigments do not synthesize carbohydrates, but in light the carbohydrate-sugar balance is altered leading on to the formation of sugars. Thus instead of forming starch as is usually the case in light starch is hydrolysed. The osmotic pressure naturally goes up and the guard cells distend and consequently the pores open.

(*b*) *Temperature.*—Temperature affects the opening of the stomata in two ways: (1) it reduces the length of the time of the opening by one-half for every 10° rise of temperature, and (2) at higher temperatures starch gets converted to maltose, thus increasing the osmotic pressure of the guard cells in consequence of which the stomata open.

(*c*) *Moisture.*—Next to light, moisture has the greatest effect on the stomatal movement. If the supply of water in the leaf reaches a certain minimum, which may cause the guard cells to loose their turgidity, the stomata will close regardless of the influence of light or any other factor.

(*d*) *Salts.*—Iljin and others have shown that salts have a marked effect upon the stomatal movement. In general, if the leaves are floated on salt solutions, by the penetration of salts into the guard cells, starch gets hydrolysed to sugar and stomata open.

Daily Movement of Stomata.—Loftfield has classified stomata into 3 main heads with regard to their daily movement:—

(1) The Alfalfa type: here the stomata are open all day and closed all night. This type of stomatal behaviour is met with in most thin leaved mesophytes e.g., pea, bean, turnip, radish, apple etc.

(2) The Potato type: the stomata here are open all day and night except for a few hours in the evening as in the potato plant. The stomata of the cabbage, onion, plantain, and pumpkin also fall under this type.

(3) The Barley type: these show no opening at all during the night and are open only for a few hours in the day. Generally the cereals come under this group.

Position of Stomata on the Leaf.—Stomata can be grouped under 4 heads regarding their position.

(1) In *Tropaeolum* and mulberry etc. they occur only on the under surface,

(2) In pumpkin, bean, and tomatoes they occur more on the under surface than on the upper,

(3) In cabbage, sunflower, corn, and pea, they occur equally on both surfaces, and finally

(4) In water-lily all are found in the upper surface.

The stomata average from 100 to 300 per sq. millimeter of leaf surface Brown and Escombe have shown that if the diameter of the opening of a stoma is of a certain length and if other stomata of the leaf are situated 8 to 10 diameters apart, the flow of gases

takes place as if there was no covered area, but that there was one continuous opening. It should be remembered that normally the total area covered by the opening of all the stomata is only about 1 per cent. of the leaf surface, the rest being covered up with epidermis and cuticle. It has been, however, recently shown that slight closing of the stomata has practically no effect on the transpiration rate. It is only when they are almost closed that transpiration is affected.

Light.

Light affects the rate of transpiration in various ways, e.g ,

(*a*) It affects the guard cells, as has been mentioned before by altering the starch-sugar equilibrium.

(*b*) It increases the temperature of the leaves.

(*c*) It increases the permeability of the protoplasmic membrane, thus reducing the resistance to the passage of water to the cell-walls.

(*d*) It induces imbibitional chances in the cell-wall colloids.

Temperature.

In general the rise of temperature increases the rate of transpiration. by simply increasing the evaporating power of the leaf. It should, however, be remembered, that different plants react differently to temperature, the transpiration rate augmenting more in some and less in others.

HUMIDITY.

Decrease in humidity increases in general the transpiration rate because the evaporating power naturally increases with decreasing humidity.

Effects of Environment on the Structure of Transpiring Organs

As water is not uniformally distributed over the surface of the globe nor is the temperature constant throughout the universe, it necessarily follows that plants have to adapt themselves to different surroundings in order to live.

Plants of the arid regions where soil moisture is very scanty and the air is hot and dry, modify their transpiration rate by some of the following ways.

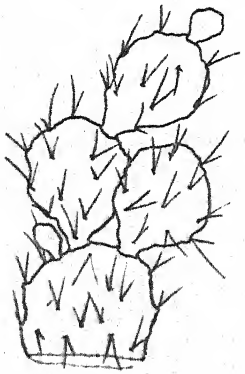


Fig. 46.—A branch of a cactus plant.

(1) *By Reduced Surface.*—A familiar example is found in the cacti. These plants have thick fleshy

stems with well developed chloroplasts, serving as assimilatory organs. The leaves, on the contrary, are small and spiny, and have lost their assimilatory power. Some times these phylloclades store up enormous quantity of water and even when removed from the parent plant can live for quite a number of years without taking up water.

(2) *By the Sinking of the Stomata.*—A familiar example one finds in *Nerium.* Here not only are the leaves thin and long—a necessary adaptation for reduced transpiration—but the stomata are sunk in cavities.

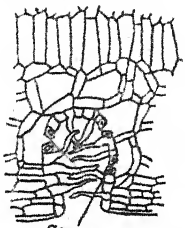


Fig. 47.—Transverse sec. of a part of **Nerium** leaf through a sunken stoma.

These cavities open as tiny pores on the surface and retain moisture in them; as the dry winds pass over the pore the moisture within remain undisturbed.

(3) *By Reducing the Number of Stomata.*—Reduction in the number of the stomata also helps to reduce the transpiration as is found in grasses.

(4) *By Thickening of the Cuticle.*—In pines the cuticle is thickened and thus the cuticular evaporation is reduced to the very minimum.

(5) *By Production of Waxy Bloom.*—Another interesting way by which plants reduce their transpiration is by the production of waxy bloom upon their cuticle. Familiar examples may be found in the cabbage, sugarcane etc.

(6) *By curling or rolling of the leaves.*—Leaves of some of the grasses have special adaptations for Xerophytic conditions. There are a number of longitudinal grooves on the upper side of these leaves. Epidermal

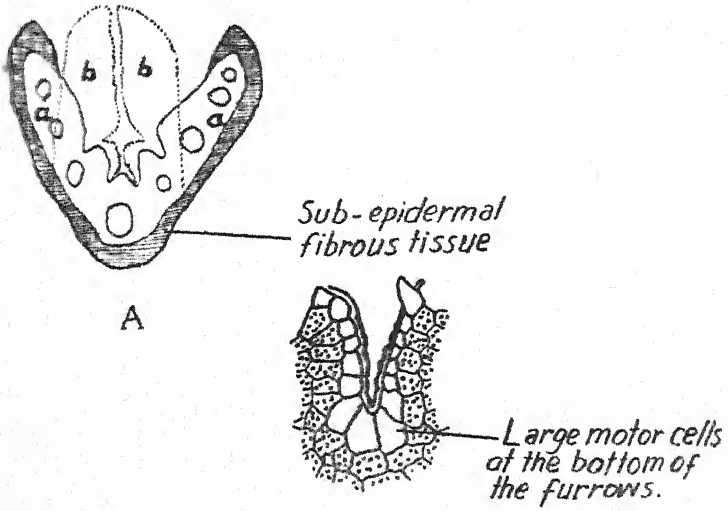


Fig. 47a.—A. Diagram of a leaf of Festuca glauca in T.S.; a-a leaf expended; b-b leaf folded up.
B. T.S. across one of the furrows on the upper surface side. (After Tschirch)

cells at the bottom of these grooves—*the motor cells*—are of larger size and their walls are thin and flexible. Under the conditions of insufficient supply of water or increased dryness of atmosphere there is a decrease in

the turgidity of these motor cells and they contract. This, as well as the simultaneous contraction of the sub-epidermal fibrous strands result in the folding or the curling of the leaves thus avoiding excessive transpiration (Figures 47A and B).

The *Alpine plants* also exhibit the characteristics

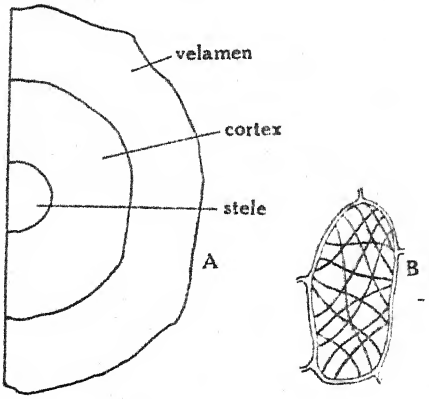


Fig. 48.—Diagram of (A) a cross section of orchid root and (B) a single cell of velamen.

of xerophytes. Due to very low temperatures even though the rainfall may be high, the plants are unable

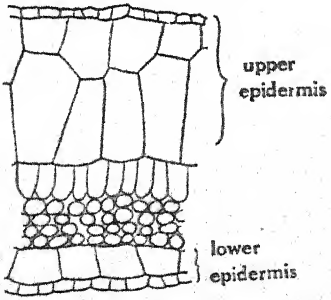


Fig. 49.—Cross section of **Begonia** leaf. The many layered epidermis serve as water storage tissue.

to withdraw water from the soil and consequently they have to conserve their water. Thus alpine soils though containing plenty of water are physiologically dry soils.

There are yet other plants like *Begonias* and orchids that possess *water storage tissues.* When there is rainfall the roots of orchids absorb water and store it up in their special storage organs called *velamen* (Fig. 48).

Relation between Transpiration and Evaporation

As has been said in a previous page that plants can regulate their transpiration rate to a certain extent by

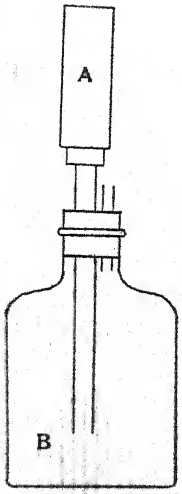


Fig. 50.—An atmometer; A, a porous pot mounted over a bottle B, containing water. Evaporation is measured by the loss in weight of the apparatus.

various means. Thus it may be useful to find out the relation between evaporation and transpiration.

There are many evaporimeters also called atmometers on the market. In their design the underlying plan is to have a porous clay cap, from whose outer surface the water keeps on evaporating and consequently withdrawing water from the inner core (see Fig. 50).

The *relative transpiration,* also called *transpiration coefficient,* is the ratio of transpiration to evaporation. The variability in the ratio gives an indication of what is termed physiological checking of transpiration.

Practical Experiments

EXAMINATION OF SOILS.

Examine the following kinds of soils:—

(1) Sand, (2) Loam from surface soil, (3) Loam from sub-soil, (4) Clay.

1. *Sand.*—It is noncolloidal and settles quickly in water. It can be wetted readily and dries quickly. On examining a few particles under microscope it is found that all the particles are separate; there is no aggregation. Some of the particles are brownish in colour and opaque and some are translucent.

2-3. *Loam.*—The soil from the surface is darker than the sub soil which is yellowish. The blackness is due to the presence of decayed organic matter of humus. The soil does not settle so readily as the sand. Also it does not dry up so quickly. The loam is crumby in nature and contains sand particles around which are aggregated loam particles.

4. *Clay.*—It is sticky and pasty when wet. It does not settle down readily in water and is of a colloidal nature, when observed under the microscope the parti-

cles are much finer than those of sand and loam and are very compact. By examining a soil suspension of clay under high power of the microscope Brownian movement can be observed.

Reaction of Soils.

1. *Ordinary soil.*—Take a little ordinary soil in a test tube. Add water and decant the fluid. Test its alkalinity and acidity. It is practically neutral.

2. *Usar soil.*—In a test tube take a little usar soil and add water. Decant and add phenolphthalein to the fluid. A red colour is produced showing that the usar soil is alkaline.

Examination of Root, Root-cap and Root Hairs.

1. Take a young seedling. Remove the soil from its roots by carefully washing them in water. Put it on a slide and examine the roots under low power. Note the root cap at the apex and root hairs on the sides a little away from the tip. Compare these root hairs with those of a water plant. The root hairs are many and not straight while in the water plant they are few and straight.

2. Examine a few root hairs under the high power. Note the wall and the protoplasm surrounding a vacuole.

Experiments on Osmosis.

1. *Exosmosis.*—Some grapes are put in strong salt solution in a dish. After sometime the grapes show shrinkage. The cell sap inside the grapes is drawn out by the stronger salt soluton in the dish.

2. *Endosmosis.*—Some dry raisins are put in pure water. After sometime the raisins swell up. This is

due to the fact that the cell sap inside the raisins being concentrated the water from the dish passes inside the raisins.

3. *Demonstration of osmosis.*—The mouth of a thistle funnel is covered with goat's bladder and tied. The funnel is inverted and sugar solution is poured inside till it reaches a short distance in the tube of the funnel. Its level is marked. The funnel is then dipped into a dish containing distilled water. After sometime it will be seen that the level of the liquid in the tube of the funnel has risen.

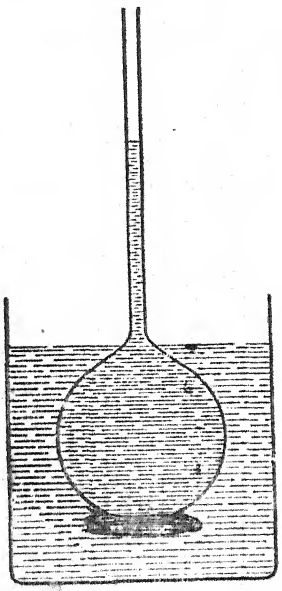


Fig. 51.—Diagram illustrating osmosis.

4. *Demonstration of osmosis by another method.*—In this experiment the rise of the liquid is shown by the rise in the mercury level in a manometer tube. See Fig. 52. The pressure developed in osmosis can be

estimated from the difference in mercury levels in the manometer.

5. *Demonstration of turgescence of the cells.*—Take three dishes. Put in dish (I) water, in (II) weak salt solution, and in (III) strong salt solution. Put in all the dishes longitudinally cut pieces of balsam stem. After some time it will be seen that the pieces in water bend towards the epidermis, the pieces in weak salt solution are practically straight while those in strong salt solution are bent towards the pith. Explain.

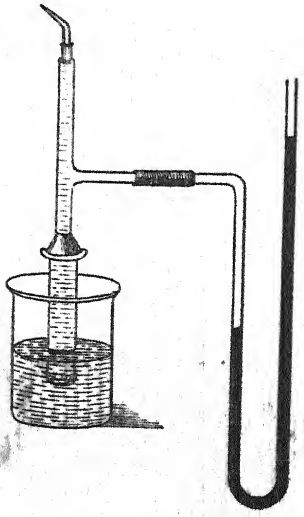


Fig. 52.—Diagram of apparatus for demonstration of osmotic pressure.

6. *Experiment to determine the amount of osmotic pressure.*—A piece of herbaceous stem about 100 mm. long having 1 or 2 mm. diameter is marked with India ink at equal distances. This piece is then put in 10 per cent salt solution for about 24 hours. It will be seen that the distances between the ink marks have shortened. The stem is now stretched by putting

weights on the scale pan of the apparatus. That which in the experiment is affected by weights, is affected in nature by osmotic pressure. Thus finding the average diameter and applying the formula $\pi r^2 h$ the osmotic pressure can be determined.

7. *Demonstration of plasmolysis.*—Peel off the lower coloured epidermis of *Rhoeo discolor* and examine under the miscroscope. It will be seen that each cell is completely filled with a reddish cell sap. Now take three other pieces of the epidermis and put them in three glasses containing salt solutions A, B and C of various strengths. Note the result and explain.

Entry of Water and Ascent of Sap.

1. *Experiment to show the formation of Pfeffer's artificial membrane.*—A crystal of copper sulphate is dropped in a solution of potassium ferrocyanide. A semipermeable membrane of copper ferrocyanide is formed which goes on growing progressively.

2. *Experiment to show the path of water through the xylem in the stem, leaf and flower.*—Place a twig of white flowered balsam in a beaker containing eosin solution. After an hour it will be seen that the veins of the leaves and petals of flowers are coloured red. Cut a T. S. of the stem of this plant and observe that only the xylem vessels are turned red.

3. Repeat the above experiment with the leaf of *Arundo.*

4. *Experiment to show the wilting of a shoot as a result of plugging the lumen of the vessels with paraffin.* —Cut a shoot of a balsam plant and dip it in a pot containing cool but melted paraffin; withdraw and when the paraffin has set remove a thin section from the cut end. The lumen then will remain plugged while the side will be freed of paraffin. Place the plant in a

Transpiration.

1. *Experiment to show loss of water due to transpiration.*—A potted plant is placed under a bell-jar. The pot is covered by a parchment paper or an oil cloth, in such a way that no water vapour escapes out of it. The bell-jar is made air-tight. After a time it will be found that small drops of water have deposited on the inner wall of the bell-jar.

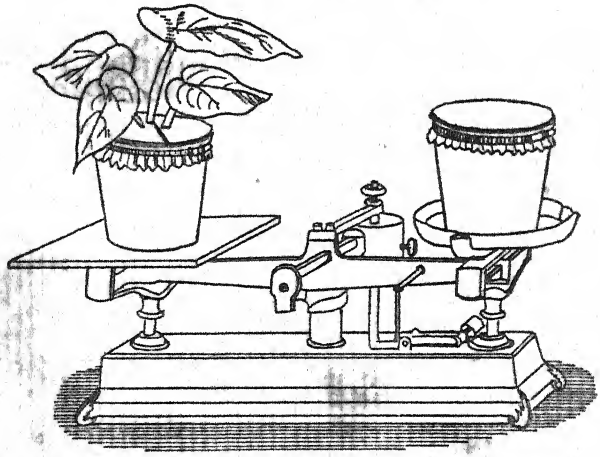


Fig. 55.—Experiment to show loss of water by the help of balance.

2. *Experiment to find the amount of water lost due to transpiration by means of a balance.*—A potted plant is placed over a balance. The pot is wrapped on all sides by a parchment paper to check evaporation. After a time the loss in the weight of the plant is observed.

3. *Suction force due to evaporation.*—To the broader end of a thistle funnel is tied a piece of goat's bladder. The funnel is then filled with water and dipped in mercury. As a result of evaporation from the surface of the bladder mercury enters the tube.

4. *Experiment to show suction force due to transpiration.*—By means of a piece of rubber tubing a leafy shoot is connected air-tight with a straight glass tube, which is then filled with water and dipped into mercury. As the water is lost in transpiration mercury enters the tube. The experiment shows the suction force due to transpiration.

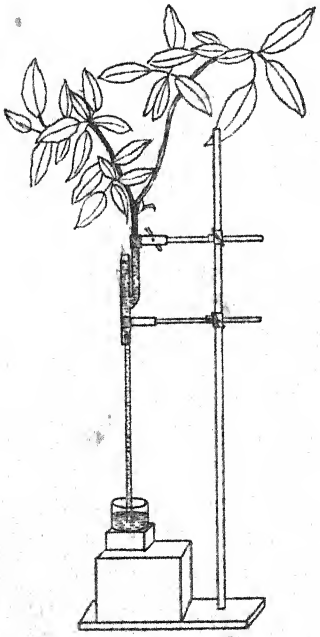


Fig. 56.—Diagram of arrangement to show suction force due to transpiration.

5. *Experiment to show transpiration by means of an ordinary potometer.*—The apparatus is fitted as shown in Fig. 56. A bubble of air is introduced in the glass tube by raising the apparatus above the level of water for a few seconds. It will be observed that the air bubble rises up.

6. *Experiment to determine the rate of transpiration by Ganong's potometer.*—The apparatus is fitted as shown in Fig. 57. Here also a bubble of air is introduced in the apparatus and its rate of movement is measured. The advantage of this apparatus is that the same bubble can be used for a number of experiments.

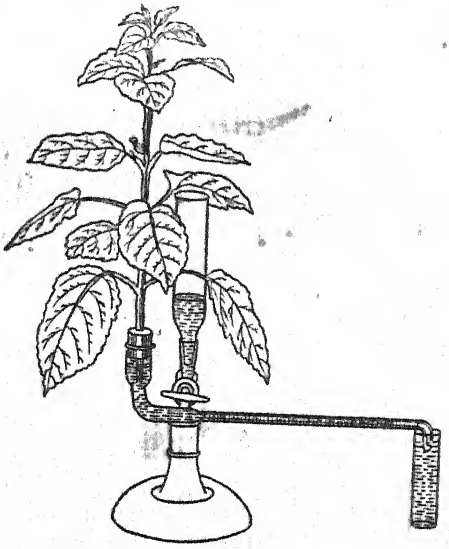


Fig. 57.—Ganong's potometer.

7 and 8. The above experiment is repeated by using Farmer's (Fig. 58) and Bose's potometers.

9. *Experiment to show loss of weight due to transpiration by a spring and horizontal microscope.*—The apparatus is fitted as shown in the Figure 59. A leafy twig is placed in water kept in a small tube. The upper end of the tube is plugged with cotton. The tube is hung by a thread attached to a spring. A pin is adjusted in the thread and viewed through a horizontal microscope. As transpiration takes place from

the leaves, the weight of the plant goes on decreasing, as a result of which tension on the spring is lowered.

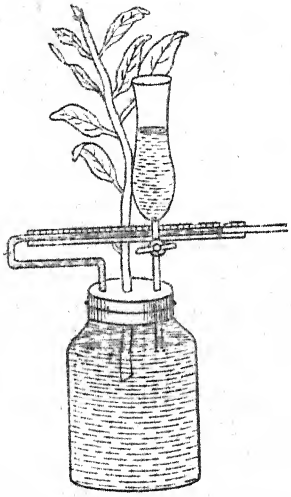


Fig. 58.—Farmer's potometer.

and the position of the pin goes on shifting upwards which is detected by the graduations in the microscope.

10. *Experiment to compare transpiration from the two surfaces of a leaf by means of cobalt chloride paper.*—Lay a dried cobalt paper on a dry glass slide. On the paper place a fresh leaf of *Nerium* and cover this with a second piece of dried cobalt paper and a second slide. Tie both the slides by a piece of cord. After an hour it will be found that the cobalt paper in contact with the lower surface of the leaf assumes a red tint, while the other retains its blue colour. Care should be taken while conducting the experiment that the hands are dry.

11. *Experiment to determine the amount of transpiration from the upper and lower surfaces of a leaf.*—

Two similar bell-jars are cemented by vaseline to the upper and the lower sides of a leaf. Within each bell-jar is placed a tube filled with known quantity of cal-

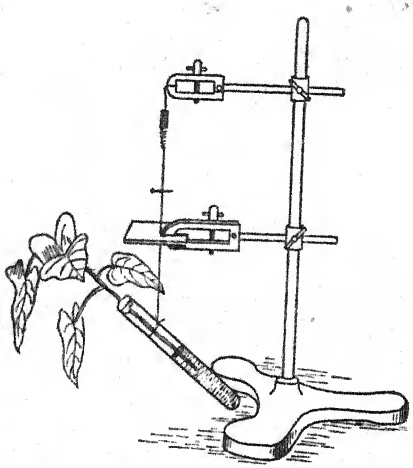


Fig. 59.—Experiment to show loss of weight due to transpiration.

cium chloride. The increase in weight of these tubes indicates the amount of transpiration from the leaf surfaces (Fig. 60).

Experiments on Stomata.

1. *Experiment to measure the stomatal aperture in leaves*

Lloyd's method.—Peel off portions of the epidermis of the leaf provided and place them in absolute alcohol tinged with congo-red. The guard cells are so rapidly dehydrated and hardened by alcohol that the stomata are not distorted in shape. The sizes of the openings of the stomata can now be measured under the microscope.

2. *Comparison of stomatal apertures by means of Darwin and Pertz's porometer.*—A small funnel

is fixed to the leaf by means of gelatin. To this funnel is attached a piece of rubber tubing which in turn is attached to one arm of a T-piece. The lower end of the T-piece dips under mercury contained in a beaker while a small-bored piece of rubber tubing with

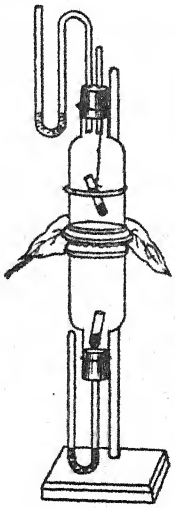


Fig. 60.—Diagram of apparatus for investigating transpiration from the two surfaces of a leaf.

a clip is fixed to the other arm. By opening the clip mercury can be sucked up to any desired height and the clip is then closed. A slight negative pressure is now created in the porometer chamber. Note the rate of fall of the mercury column. The stomatal apertures of different plants can be compared by using the same apparatus with different plants placed under similar conditions.

3. Types of daily movements of stomata.

(a) Alfalfa type.—Stomata open all day and closed all night as in pear, apple, radish, bean etc.

(*b*) *Potato type.*—Open day and night except for a few hours in the evening as in plantain and pumpkin etc.

(*c*) *Barley type.*—Show no opening in the night and are open for a few hours in the day as in cereals.

4. *Position of stomata.*

(*a*) *Only on under surface.*—Apple, peach and mulberry.

(*b*) *More on under than on upper surface.*—Pumpkin, potato, bean.

(*c*) *Equally on both surfaces.*—Pea.

(*d*) *Only on upper surface.*—Water lily.

5. *Devices of reducing transpiration.*

(*a*) Position of stomata in the leaves: Cut the leaves of the following, examine and sketch:—

Balsam.—Stomata on the lower surface flush with the epidermal layer.

Pinus and Carnation.—Sunken stomata due to xerophytic conditions.

Nerium.—Stomata in grooves with numerous hairs on the sides of the grooves.

Andropogon.—Two guard cells are fused on one side of the stomata and two on the other. It can easily be seen on peeling off the epidermis.

(*b*) *Waxy bloom on sugarcane nodes.*—Note the white powdery mass on the nodes and leaves of the sugarcane which reduces transpiration.

(*c*) Examine the phylloclades of *Muehlenbeckia* and *Cactus* and note that the flattened structures are really stems but have taken the function of the leaves. Leaves are spiny and reduced in *Cactus*. It is also a device for reducing transpiration.

CHAPTER VII

INTAKE OF SOLUTES BY PLANTS

In the foregoing chapter it was told that the plant cells had semipermeable membranes and it was to the cell-wall—protoplasm—vacuole system that the cells owed their turgid condition. The cell membranes are, however, not absolutely semipermeable, but allow the passage of solutes through them. Osterhout has shown that the inorganic salts readily penetrate the protoplasmic membrane and enter the vacuole of the cell. He experimented with *Dianthus barbatus* which contains lot of oxalic acid and grew it in distilled water. After a few days when root hairs appeared he transferred some plants to a weak solution of calcium nitrate and kept back some on distilled water. After a few hours when the root hairs were examined under the microscope crystals of calcium oxalate were seen in the root hairs that were in calcium nitrate solution while no crystals appeared in the control. This conclusively showed that calcium entered the root hairs in ionic form.

It has been generally considered that inorganic salts enter the cells in the dissociated form. Recently, however, Osterhout (1929) has shown that certain compounds enter the plant cells without undergoing dissociation.

Generally speaking the protoplasmic membranes of plants are permeable to practically all the inorganic

solutes and up till recently it was generally assumed that it was a simple process of diffusion whereby the entrance of the solute was from the higher concentration in the soil to the lower in the cell sap. If after the entrance no change took place then equilibrium would be established and no further penetration could take place. But if the solutes were removed from the cell or chemically combined, precipitated or absorbed thus rendering them osmotically inactive, then the flow of the solutes will continue.

Recently, the examination of the uncontaminated sap of individual cells have shown that in many cases the concentration of the osmotically active solutes is higher than the surrounding medium. This will mean that the intake of the solutes is not a simple process of diffusion.

Osterhout (1922) working with *Valonia* found the mineral contents in the cell sap and in sea water as follows:

	Concentration in sea water	Concentration in cell sap
Chlorine	19·6	21·2
Sodium	10·9	2·1
Potassium	0·5	20·1
Calcium	0·45	0·7
Magnesium... ...	1·31	Trace
Sulphates	3·3	0·005

Sodium, magnesium and sulphates are much less in concentration inside the cell than in sea water. Chlorine is nearly as high as sea water while

potassium is 50 times more concentrated in the cell sap than in sea water. As practically all the potassium is in the soluble form of chlorides its presence cannot be attributed to removal from the metabolic centre of chemical changes.

Thus it is a well established fact that the plasma membrane surrounding the protoplasm is not perfectly semi-permeable and allows the inward passage of various substances. In reality this phenomenon is of utmost importance for the balanced metabolism of a living cell. During the latter part of the 19th century de Vries, Klebs and others showed that when plant cells were put in a solution of substances like glycerol, cane sugar etc. first plasmolysis occured due to exosmosis but after some hours by gradual inward passage deplasmolysis took place and the cells once more recovered. The physiological sensitivity of the plasma membrane, as expressed by differential permeability towards substances in solution, has been explained by various theories put forward by different sets of workers. A brief account is summarised below.

Differential Membrane Action in Plants.

Sieve theory.—This is also known as Ultrafiltration theory. Traube (1879), Ruhland (1914) and Seifriz (1936) put forward the view that the plasma membrane acts as a sieve or an ultra-filter. Thus solute particles beyond a certain size cannot enter in a cell. According to Seifriz the size of the pores can change and adjust to suit the surrounding conditions. That the various

dyes which cannot pass through artificial parchment membrane are absorbed by living cells through their plasma membrane has been the strongest evidence in favour of this theory.

Later on it was shown that in many cases large molecules as those of alkaloids were permeable and small molecules of amino acids were impermeable to the same membrane. This fact stood against the sieve theory and Brooks (1930) modifying the theory said that plasma membrane consisted of mosaic of anion permeable and cation permeable areas which were of the nature of charged porous films. This explained the rapid diffusion velocity of one type of ion as compared to the other type.

Amphoteric nature of the protoplasm is considered by some to be responsible for the intake of solute by the plants. At a certain pH value of the medium known as "Isoelectric point" protoplasm is neutral but on the acid side of this value it behaves as a cation and unites with anions while on the alkaline side it behaves as an anion and unites with cations. Lapique (1925) held the view that protoplasm during its circulation within a cell happens to contact both the exterior medium which is on the alkaline side of its isoelectric point as well as the cell sap which is on the acidic side of the isoelectric point. So with cell sap it releases cations and combines with anions and with the exterior medium it combines with cations and releases anions. This process continues in all the cells, from the exposed absorbing surface to the interior parts and maintains

the absorption and transmission of ions from the external solution to the inner tissues.

In the opinion of Casale (1921) the ectoplasm of an exposed absorbing cell releases hydrogen ion to the external solution. Thus a difference of potential is set up between the two. To equalise the charges, the external solution yields cation to the ectoplasm. This cation may have been transmitted by a distant part of the external solution by the process of the replacement of charge and not actual movement. Thus the ionic exchanges between the external solution, cytoplasm and the cell sap become responsible for the intake of solutes.

(N.B.—*The reader is expected to have an elementary knowledge of hydrogen ion concentration. Those who are ignorant are advised to read Appendix B*).

Colloidal theory.—This may be considered only as a corollary to the sieve theory. Protoplasmic membrane is of the emulsoid colloidal type and various investigators have suggested that the changes in viscosity, phase inversion or electrical reactions of this type of membrane might bring about changes in the permeability resulting in selective absorption of solutes.

According to Breazeale (1923) attraction of colloids for ions is responsible for the intake of solutes in plants. If some ion is taken from a colloidal compound within the plant and used in building up a permanent tissue then the remainder of that colloidal compound loses its electrical equilibrium and carries either positive or negative charge. This charge is transmitted by re-

placement and not any bodily movement till it reaches the epiblema of the root, where to regain the electrical equilibrium the same ion, which has been used up within, is taken up from the external solution.

Lipoid theory of the plasma membrane.—Overton (1895) has been the chief supporter of this theory. He concluded that plasma membrane was composed of fatty or fat like substances, collectively known as lipoids and only those solutes which were soluble in lipoids were permeable to these membranes. Overton worked on aniline dyes which were soluble in lipoids. When these dyes were applied to the cells no plasmolysis occured and overton concluded that the dyes dissolved in the lipoid of the plasma membrane and entered in the cell without causing any plasmolysis.

But others have pointed out that the occurence of plasmolysis in the beginning, cannot be a criterian of impermeability of that solution because slow penetration of the same will bring deplasmolysis later on.

Further the chief objection to Overton's theory is that inorganic salts which are insoluble in fats enter in large amounts in the living cells.

Nathansohn went a step further and suggested the *mosaic theory of the plasma-membrane.* According to this the plasma-membrane has a mosaic of lipoid and protein material. Thus the simultaneous intake of lipoid soluble and protein soluble substances may be explained but the main objection to the Lipoid theory regarding the entry of inorganic salts remains valid against this theory also.

The Chemical theory.—This theory incorporates, by far, the most accepted view. Upholders of this theory consider that the plasma membrane combines chemically with the substance to which it is permeable. And this chemical union is reversible, so that the compound formed between the membrane and the solute in question liberates the same solute again on the inside of the membrane.

The chemical theory is further supported by researches on the temperature coefficient of permeability. This coefficient (Q 10) is meant to indicate the increase in the permeability of the membrane for each 10° C increase in temperature. The values obtained for Q. 10 are relatively low (1 – 1·5) for physical reactions as compared to the values (2–3) for chemical reactions. Data accumulated in connection with the Q 10 of permeability experiments with plants indicate that this process in the living cells cannot be exclusively a physical one.

Some have suggested that various salts accumulate in the cell sap by greater expenditure of energy made available due to increased aerobic respiration. This is because cells must do work in absorbing the solutes. In this way movement of ions from low concentration in the external solution to higher concentration in the plant cells becomes possible. The process is similar to the glandular secretion in animals where it is supposed that dilute compounds are taken up from the blood and secreted in higher concentrations.

Keeping in view the varied and perfect mechanism

of ion absorption in the living cells, none of the above theories are complete to explain this phenomenon. They are mostly limited to the range within which the observations have been made in each case.

Antagonism of Salts and Balanced Solutions

It was Loeb (1900) who, while working on the development of the marine fish *Fundulus,* first showed that solution of a single salt such as sodium chloride, though having the same concentration as that of this salt in sea water, had an injurious effect on the organism. But if to this solution a small quantity of the salt of a bivalent metal such as calcium, magnesium or even lead is added then the development goes on as usual. Thus the presence of a second substance dissolved in the solution of the first reduces the harmful action of the toxic substance. Since then it has been shown for all marine organisms that they perish if put on a single salt solution of the same concentration as that of sea water. The term *antagonism* signifies the hindrance which one salt has upon the toxic action of another. Generally speaking there exists an antagonism between the monovalent and divalent cations, though slight antagonism is also seen between cations of the same valency. It is thus possible to mix two or more substances in solution, which singly may be toxic but which in a mixture, due to antagonism reduce the harmful action of each other. Such a solution is called a *physiologically balanced solution.* Such solutions need not be good nutrient solutions, as they may not

contain all the salts necessary for the growth of a plant. A balanced solution simply indicates that the various salts are present in such proportions as to render individual toxic effects impossible. On the other hand a nutrient solution has all the salts necessary for plant growth in such proportions as to render it a physiologically balanced solution. As to how the toxic action of a single salt is reduced is as yet vague. But it is suggested that the antagonistic cation hinders the entry of the toxic salt. Osterhout to whom we owe a lot in this field of physiology has shown that marine plants if kept in a solution of sodium chloride isotonic with sea water die within a very short time. But if only a very slight amount of calcium chloride is added to this then the toxic action is reduced to a considerable extent. But if to this mixture some potassium chloride is also added then the plants live much longer and when magnesium chloride and magnesium sulphate are further added to this mixture, then the marine plants can live as long as they do in sea water.

Practical Experiments

Experiment to demonstrate selective absorption.— Put some alga in test tubes containing solutions of neutral red and methylene blue. Examine after a few minutes. It will be seen that the alga in neutral red has turned light red while the one in the methylene blue is still green. It shows that the alga absorbs neutral red but not methylene blue. The experiment demonstrates selective permeability of the protoplasm.

CHAPTER VIII

TRANSPORT OF FOOD MATERIALS

The evolution of food conducting and water conducting tissues is correlated with the evolution of leaves. In the Thallophytes and the Bryophytes there are no well defined conducting strands for the simple reason that the tissues of these groups are not so well defined. It is obvious that in the case of the highly evolved plants the food manufactured in the leaf must be translocated to other parts for its growth and future use. In many cases food has to travel great distances necessitating fundamental changes in the design of the conducting strands.

Then again, the peculiar needs of each individual plant is distinct and has to be satisfied. A germinating seedling needs food at the growing tip of the plumule and at the same time the tip of the radicle requires food. On the formation of the first assimilatory leaves the entire course of the supply channels are altered and new supply routes from the leaves to the growing points are opened. On the other hand, in a corn plant when the ears are formed the food manufactured in the leaves travels upwards or downwards, as the case may be, to nourish the young fruits. In all cases, however, the food in the leaves first travels down to the node from whence it either goes up or down.

In mature plants where growth has ceased all the food goes downwards and accumulates for the next season's growth. After the winter cessation of growth, when it again bursts forth into activity, food is carried from the branches, trunk and roots to the growing buds. But when leaves unfurl, they begin to manufacture their own food and thus alter the supply routes to these growing points.

We have now to study these supply routes and the way they function. Malpighi (1679) was the first to have noticed that crude sap ascended through the wood to the leaves where it was in some way changed and then passed backwards through the outer tissues to regions of storage or growth. He was led to this conclusion by his brilliant idea of conducting ringing experiments which even today forms an essential experimental method, to the study of this problem. Hales (1727) also carried out ringing experiments and suggested a flow and ebb of sap through the wood. Knight (1801) showed that tissues below the ring failed to grow unless a shoot or leaves were present, thus proving that phloem was responsible for the downward movement. He showed also that a coloured solution travelled up through the xylem. The conclusion as to the upward movement through the xylem was substantiated by the facts that (1) large quantities of water are absorbed from the soil and through the xylem vessels conducted to the leaves and there evaporated, (2) coloured solution applied at one end are quickly carried through the wood to the transpiring

leaves and (3) cut stems often exude a sap containing various salts and sugars. The arguments for the downward movement of food material were based upon ringing experiments. The bark of a plant is removed up to and including the phloem in the form of a ring. When such experiments were performed food materials accumulated above the ring, showing thereby that they could not pass downwards through the xylem. Such plants also showed more growth above the ring.

Dixon and Ball (1922) found sugars as well as proteins in the wood. According to them ringing experiments are not of much value as due to ringing (1) the surface of the wood may be injured and (2) air may enter the wood. Again the rate of depletion of the carbohydrates in potato tubers show that it is very much faster than what the sieve tubes can carry. A petiole of *Sambucus nigra* was split longitudinally and the portion that was left attached to the pinnae was dipped in eosin solution. The solution was drawn up into the veins of the pinnae from the bottom. In the next stage when the solution had reached the tip, it worked its way downwards from the other side of the pinnae. From this he concluded that the transport in both directions takes place through the xylem.

Curtis (1925), however, maintains that both upward and downward movements of sugars, nitrates and ash take place through the phloem. He ringed his stems and protected them from drying with paraffin. From his experiments he concluded that there was considerable interruption of flow to the growing regions,

while only a small strip of xylem was enough to transport adequate quantity of water.

He also divided stems whereby water was supplied to the top by one set of roots and nitrogenous materials by another, whereby he showed that nitrogenous materials were not transferred through xylem. But if xylem was connected by a strip of phloem then normal translocation took place.

Weevers (1923) used variegated shoots of *Acer*. With the complete ringing of the yellow shoots no growth took place, as shoots were unable to manufacture their own food. But with partial ringing growth was visible. Ringing the green shoots had no effect upon growth. Thus showing that phloem is the channel for transport of food.

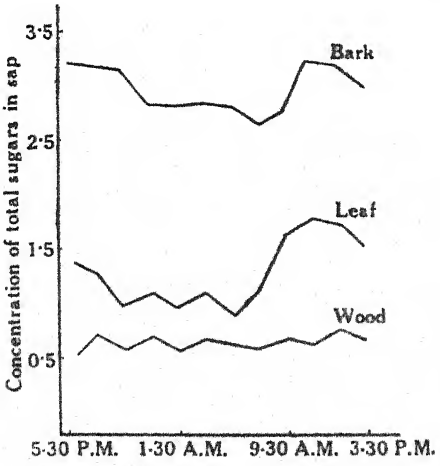


Fig. 61.—Graph showing concentration of sugars in sap from bark (including sieve tubes), leaf, and wood.
(Redrawn from Mason and Maskell).

Mason and Maskell working on cotton plants made an analysis of bark, leaf and wood separately. The results are graphically given on page 167. The graph shows that the movement of the carbohydrates in the leaf and bark synchronizes; (sieve-tubes are included in the bark) indicating that phloem is the channel for the transport of food.

A theory put forward by Münch (1926) and by Crafts (1931) tries to satisfy both the previous views. They show that both upward and downward transference of organic food material takes place through phloem and the upward movement of salts through the xylem. As all the above theories are mostly based on ringing experiments it may be advantageous to know

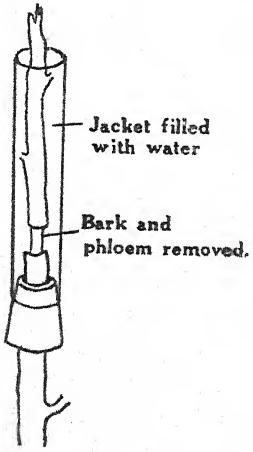


Fig. 62.—Diagram showing the arrangement of a ringing experiment.

as to how such experiments are performed. For these experiments a plant is taken that has collateral bundles, i.e. phloem on one side only. Then with a clean sharp

knife two circular incisions are made at a distance of one to two inches from each other. The bark is then carefully removed (Fig. 62). Care should be taken while making the incisions not to injure the hard xylem within. After a few days the carbohydrate contents above and below the ring are examined.

Some criticism has been levelled against this procedure for due to drying up of the exposed xylem vessels the experiments may be vitiated. To overcome this difficulty a glass tube filled with water is jacketed on the ring.

Practical Experiments

1. *Ringing of the twig.*—Two cuts about an inch apart are made in the lower part of a *Croton* twig. The soft outer tissue of the stem between these cuts is removed so as to leave only the hard wood portion of the stem for this distance. The twig is then put in water. After a few days it will be noticed that below the injury the development of buds and new roots is very slow whereas above it new roots are rapidly formed. This shows that food passes down chiefly through the soft outer region (phloem) of the stem.

2. Cut a L.S. of *Coccinia* stem. Treat the section with Millon's reagent. Put a cover slip over the section and warm gently. Protein granules are seen coloured red in the sieve-tubes.

3. Cut a T.S. of the *Cucurbita* stem. Note the bicollateral bundles. Examine the sieve-tubes and sieve-plates. Treat the section with iodine solution. The starch grains in the sieve-tubes and sieve-plates are stained dark blue.

The experiments 2 and 3 show that the protein and starch are transported through the phloem tissue.

CHAPTER IX

GROWTH

A permanent and irreversible increase in form is growth, *i.e.*, growth is the final expression of a successful metabolism. According to F. F. Blackman it is the "Finished product of the metabolic loom". A living cell must be supplied with both food and energy for it to grow. Thus both the anabolic and the catabolic sides of metabolism are necessary. It is obvious, however, that the anabolic side of metabolism should be in excess of the catabolic.

THE STAGES OF GROWTH

The Growth of Unicellular Plants.

One of the best material for the study of growth is the brewers' yeast. If a few yeast cells are put in a mineral nutrient solution containing sugar they start growing. When a definite size is reached a daughter cell is budded off. The small daughter cell now starts growing till finally in its turn it buds. The time taken for a cell to grow till it reaches the budding phase is called the *generation time*. This varies with varying external conditions. But in normal cases the generation time is about an hour.

The divisions of all the cells in a culture solution will, however, not be simultaneous as all the cells will

not be of the same age. Thus, while some cells will be growing others will start dividing. On an average,

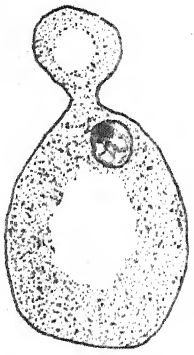


Fig. 63.—An yeast cell.

the total number of cells in the solution will be doubled at the end of each succeeding generation time. This unrestricted growth of the colony will go on till the food supply becomes limited when it will slow down and ultimately cease altogether.

We thus see that there are three stages in the growth of free cells:—

(1) The stage of the division of the cells at which they are multiplying fast.

(2) The period of enlargement during which increase in size takes place before they can divide.

(3) The senescent phase in which for one reason or another an increasing number of cells become inactive.

THE GROWTH IN HIGHER PLANTS.

In the vascular plants all the cells do not participate in growth. The greater part of the plant consists of mature cells which have ceased to grow. Here growth takes place only at the meristems *e.g.*, apices of stems, root tips, cambium and, in the case of monocotyledons, at the base of the internodes and at the sheathing leaf bases.

The cells of the meristematic regions are, as a rule, small and have no vacuoles; they are thus completely filled with protoplasm. The nuclei are comparatively large and are centrally located. The cell walls are very thin and, according to Priestley (1929), are the channels of transport for the food supply to this tissue. Here soluble proteins are carried from other parts of the plant and these are synthesized into living protoplasm. When the mass of the meristematic cells has reached a certain point, cell divisions follow. New cell-walls are now laid. This is the first stage *viz.*, *the formative phase of growth.*

The second stage of growth now follows which is *the phase of enlargement.* This stage is characterised by the appearance of vacuoles in the protoplasm, which get filled with a watery content, the cell sap.

At first they are small, enlarging rapidly later on. The process of cell elongation is due to the osmotically active substances present in the cell sap. These attract water resulting in an increased turgor of the cell, which consequently enlarges. This goes on till, due to the dilution of the cell sap, the osmotic pressure

decreases and finally equals the inwardly directed wall pressure. When this stage is reached the suction pressure becomes zero and elongation stops.

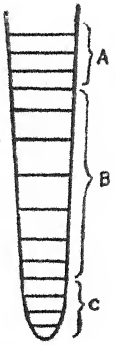


Fig. 64.—Diagram showing growth regions of a root tip; A, region of maturation; B, region of enlargement; and c, meristematic region.

Recently, Ursprung has shown, however, that during the period of elongation the cells, on the contrary, have lower osmotic pressures than the maturer cells; but they have an increased suction pressure. This can only happen if the turgor pressure decreases by a rapid growth of the cell-wall. This has been shown to be the case by many. In this second stage of growth the increase in the amount of the protoplasm is replaced by an augmented growth of the cell-wall. This causes a diminution of the turgor pressure with the consequent increase of the suction pressure causing the elongation of the cell to take place.

This is now followed by the third and last stage of growth, *e.g., the phase of internal differentiation*. The

thin and stretched wall now grows in thickness. It may now become a sieve-tube, a tracheid or any other of the numerous types of cells that compose a plant. The internal factors that control this differentiation remain unknown at present.

Growth Regulating Substances

During recent years it has come to be generally considered that the growth of plants is promoted by certain substances other than the inorganic and organic substances necessary for plant life. These are known collectively as growth regulating substances, because when applied in suitable manner and in the correct amounts, they will increase, inhibit or otherwise alter in various ways the subsequent growth of plants so treated. This group of substances includes the plant hormones, vitamins and auxins. The term *plant hormone*, when used in its restricted sense is a substance which being produced in any one part of the organism is transferred to another part and there, influences a specific physiological process. According to this interpretation it does not include many synthetic substances which have not been shown to be plant products yet have a physiological activity similar to that of the natural substances. *Plant hormones*-synonymous with growth regulating substances—might be defined as organic substances other than the traditionally recognised energy supplying substances, which regulate physiological functions in plants. This definition includes synthetic as well as natural

substances, vitamins as well as hormones, growth inhibitors as well as growth promoters. Kögl and Haagen Smit on the other hand suggested the term '*auxins*' for such substances which bring about growth by elongation and which can be measured by the curvature of the *Avena coleoptile*. Hence naturally occuring as well as synthetic substances may be *auxins* as long as they bring about growth by elongation.

When seeds of cereals e.g., wheat, barley, oats etc. germinate they send up a tender hollow spike called the coleoptile inside which are the first true leaves. This coleoptile is very sensitive to external influences. If it is grown in dark and exposed to sunlight for a fraction of a second it will curve towards the direction of light. Boysen Jensen discovered that the coleoptile of oats loses its power of responding to the stimulus of light when its tip is cut off and this power is restored by replacing the cut off tip on the headless, coleoptile. Paál confirmed Boysen Jensen's findings and obtained the first indication that the tip of coleoptile normally produces certain growth regulating substances. These, when formed at the tip, descend in the growing organs causing them to stretch. When the tip is cut off then the elongation of the lower portion decreases considerably and is accelerated only when those tissues are regenerated. If however, the severed growing tip is fixed again on top by means of water or gelatin the elongation of the cells increases. This shows that these growth regulating substances can pass both through water and gelatin. It has been further shown that when cut ends of growing tips

are placed on gelatin for some hours and then the gelatin is put on the severed stump rapid elongation of the cells takes place.

In addition to the coleoptiles of the seedlings of various grains these substances have been obtained from other plant sources e.g., seedlings of various other species of plants, buds, root tips, leaves, green algae, various fungi and bacteria.

Chemistry of Auxins

These substances have actually been isolated and identified by Kögl and his coworkers. Kögl who first suggested the term "auxins" for these substances distinguished three auxins—auxin *a*, auxin *b* and heteroauxin. Auxin *a* has the chemical formula $C_{18}H_{32}O_5$, auxin *b*, $C_{18}H_{30}O_4$ and heteroauxin, $C_{10}H_9O_2N$. All these three substances were found to occur in urine from which they were first isolated by Kögl.

Besides these three auxins Hitchcock, Zimmerman and Wilcoxon showed that similar growth regulating properties are possessed by numerous synthetic organic compounds e.g., indolebutyric acid, naphthalene acetic acid etc.

Mode of Action of Auxins in the Elongation of Plant Cells

Elongation of the plant cells ultimately is caused by water uptake. This water uptake may either be due to increase in the suction pressure produced by an action of the auxin in the cytoplasm which will eventually result

in increasing the osmotic concentration, or the auxin directly increases the elasticity or plasticity of the cell walls leading to an increase in suction pressure without the need for an increased osmotic concentration.

Recent Developments in the Knowledge of Growth Regulating Substances

(*i*) *Rooting of cuttings.*—During the past two decades the growth regulating substances have been variously used for modifying vegetative and reproductive growth of the plants. Thus by the application of some suitable growth regulating substances root formation may be initiated with greater certainty on the cuttings. Plants, which are hard to root can often be induced to root by auxin treatment and thus this becomes an easy source of vegetative propagation of horticultural and ornamental plants. Besides this, if seeds are treated with growth promoting substances during the time of germination, they will in many instances react like stems and produce extra roots which subsequently enable them to develop more rapidly than normal. Although auxins promote the formation of roots the actual growth of the formed roots is rather prevented by it. Recently it has been suggested that auxins in extremely low concentration may accelerate root growth. Thus the difference in the behaviour of stems and roots towards these substances is a matter of optimum concentration only.

(*ii*) *Parthenocarpic fruit formation*—Similarly fruit formation without pollination (parthenocarpy) may also be artificially initiated with the help of these hor-

mones. In this process the stamens are removed from the flowers and the style and stigma cut off. To the cut top of the ovary a drop of hormone solution is applied. This starts growth in the ovary without the sexual process and seedless fruits are formed.

(iii) Bud inhibition.—The development of buds is another important phenomenon in the life of the plant that is controlled by growth regulating substances. When a growing shoot is pruned it is usually seen that the first bud below the cut surface sprouts very quickly. This bud could not develop earlier because the rapidly growing apical bud prevented it. The explanation for this suppression of the growth of the lateral buds is this that when buds develop they produce far more auxin than is needed for their own growth and the excess diffuses down into the stem below. There it has the curious property of preventing the development of younger buds either directly or indirectly. This property of growth regulating substances is being widely used to prevent shoot development in Potato and delaying the development of flower buds and leaf buds, to prevent them from growing out when there is a danger of frost damage.

(iv) Prevention of preharvest fruit drop.—Somewhat similar to the prevention of lateral bud development is the prevention of preharvest fruit drop. The fruit drop is caused by a premature development of an abscission layer, which is a specialized layer of cells, situated at the base of every leaf and fruit. It was found that the development of abscission layer could

be controlled by the use of growth regulating substances in somewhat the same way as it prevents the development of cell layers in growing buds and thus it delays the dropping of the fruit. These substances although they prevent preharvest fruit drop do not delay the ripening processes which proceed normally or even at an enhanced rate.

(*v*) *Weed control.*—Some of these substances have the curious property that they are toxic to certain plants and harmless to others. Fortunately they are toxic to weeds and harmless to cereals and thus tremendous interest has developed within the last 3 years in the use of such substances for weed control.

The Nature of Growth Curves

It was Sachs who on the basis of growth of plants or parts of plants was able to establish the law of *the grand period of growth.* According to this the growth rate is at first slow, then it rapidly increases, and finally ceases altogether. The measurement of the growth can be represented graphically by two distinct curves, viz., S-shaped curve which gives the change in the total length of the plant or a part of its organ, or by a curve of increment. These are shown graphically in figures 65 and 66.

The S-shaped curve (Fig. 65) shows that the increase of the total length of the plant is at first slow then it rapidly increases till finally it assumes a horizontal

position which means that the maximum elongation has been reached. While the curve of increment starts

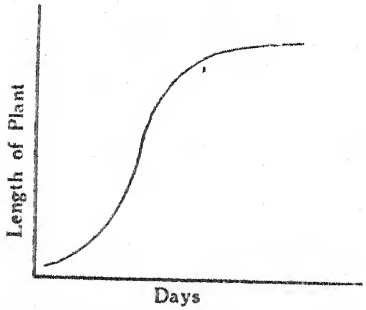


Fig. 65.—Curve showing elongation of plants.

from zero and then rapidly rises to an optimum peak and finally comes down to zero (Fig. 66).

V. H. Blackman has attempted to give a mathematical formula to this process. He applies the *compound interest law*. Here the amount of money accumulated at the end of a certain period will depend upon

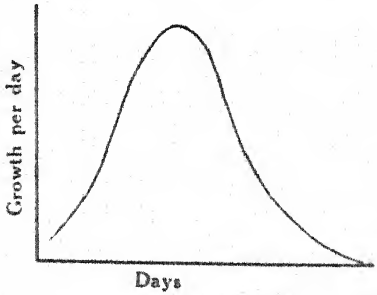


Fig. 66.—Curve illustrating the rate of increment in growing organs.

the intitial capital, the rate of interest and the time the money is accumulating. Thus the dry weight of a plant will depend upon the dry weight of the seed, the

percentage increase in the dry weight and the time during which the plant is increasing in weight. To find the percentage and growth per day Blackman applies the formula, $A = ae^{rt}$

where A is the final weight; a, the initial weight; r, the average rate of interest; t, the time, and e, the base of natural logarithms. Changing this formula to a logarithmic one and converting the natural logarithm to a decimal one, one obtains as follows:—

$$A = ae^{rt}$$

or $$A/a = e^{rt}$$

taking the logarithm to the base e we get $\log_e A/a = rt$

reducing it to base 10, we have $\log_e 10 . \log_{10} A/a = rt$

i.e. $2{\cdot}3026 \log_{10} A/a = rt$

From this it is easy to compute r, all other factors being known.

Blackman's law, however, can only apply to the first phase of the growth rate which is the phase of rapid growth. In the second phase where retardation of growth rate sets in the law cannot be applicable, and as it is difficult to determine the exact beginning of the second phase, his law can only be applicable in a very limited way.

Robertson, however, puts forward the view that the growth rate is of the nature of monomolecular autocatalytic reaction. An example of such a reaction is found in the hydrolysis of ethyl acetate to give acetic acid and ethyl alcohol.

$$CH_3CO.O.C_2H_5 + H_2O \rightarrow CH_3COOH + C_2H_5OH$$

The acetic acid formed acts as a catalytic reagent and thus the rate of the reaction goes on gathering speed. Robertson applying the above case to growth concludes that in any particular growth cycle the greatest increase in weight or volume in any unit of time takes place when the total growth cycle is half completed. Such a growth cycle will obey the following formula

$$\log \frac{x}{A-x} = k\,(t-t_1)$$

where x is the length of the plant in mm. at the completion of t days; A is the total length of growth attained during the cycle; k is a constant of the cycle; and t_1 is the time at which the growth rate is fastest, i.e. when the cycle is half completed.

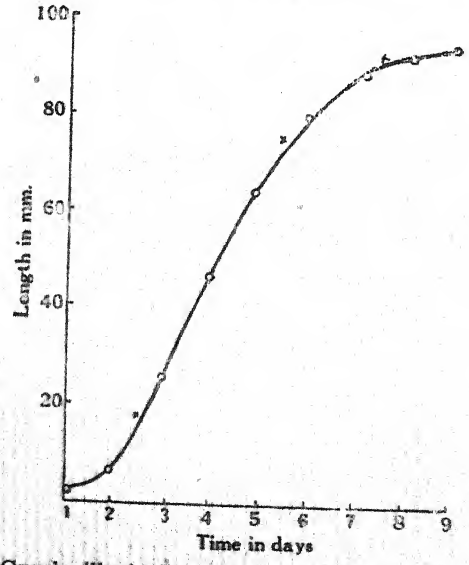


Fig. 67.—Graph illustrating Robertson's formula of growth.

Taking a hypothetical case of the growth of a seedling let us assume that the following observations were made:

t	1	2	3	4	5	6	7	8	9
x	2	7	27	47	65	80	88	93	94

where t represents time in days and x represents the growth in mm. in time t.

This is graphically shown in Fig. 66.

Let us now test whether Robertson's formula holds good. First the maximum growth rate is ascertained as follows:—

$$\text{Log } \frac{x}{A-x}=k\,(t-t_1) \text{ i.e. } \frac{1}{k}\ \log\ \frac{x}{A-x}+t_1=t$$

$$\text{Let } \frac{x}{A-x}=z \text{ i.e. } \frac{1}{k}\ \log\ z+t_1=t \qquad (i)$$

$$\therefore\ \frac{dz}{dx}=\frac{(A-x)+}{(A-x)^2}, \text{ substituting the value of } A$$

from the above observation chart, i.e. 94, we have

$$\frac{dz}{dx}=\frac{94}{(94-x)^2}$$

$$\text{Also } \frac{dt}{dz}=\frac{1}{k}\cdot\frac{1}{z}=\frac{1}{k}\cdot\frac{94-x}{x}, \text{ from } (i)$$

$$\therefore\ \frac{dt}{dx}=\frac{dt}{dz}\cdot\frac{dz}{dx}=\frac{1}{k}\cdot\frac{94-x}{x}\cdot\frac{94}{(94-x)^2}=\frac{1}{k}\cdot\frac{94}{x(94-x)}$$

Velocity of growth say v is $\frac{dx}{dt}=\frac{1}{\frac{dt}{dx}}$ i.e. $v=k.\ \frac{x(94-x)}{94}$

$$\text{For a maximum } \frac{dv}{dx}=0$$

$$\therefore\ \frac{k(94-2x)}{94}=0 \quad \text{or } 94-2x=0$$

$$\therefore\ x=47$$

i.e., growth rate of the plant is most rapid when the plant measures 47 mm. which is half the total growth in a cycle, and this is on the completion of the fourth day, i.e. $t_1=4$.

Now the value of the constant k is arrived at as follows.

Robertson's formula being $\log \frac{x}{A-x}=k(t-t_1)$, substituting the value of A and t, we have

$$k=\frac{1}{t-4}\log\frac{x}{94-x}.$$

Let us now ascertain the values of k when $t=1, 2, 3$ etc.

Days	Growth	$k=\frac{1}{t-4}\log\frac{x}{94-x}$
1	2	·5547 $\left[=\frac{1}{1-4}\log\frac{2}{94-2}\right]$
2	7	·5472 $\left[=\frac{1}{2-4}\log\frac{7}{94-7}\right]$
3	27	·3947 $\left[=\frac{1}{3-4}\log\frac{27}{94-27}\right]$
5	65	·3505 $\left[=\frac{1}{5-4}\log\frac{65}{94-65}\right]$
6	80	·2614 $\left[=\frac{1}{6-4}\log\frac{80}{94-80}\right]$
7	88	·3888 $\left[=\frac{1}{7-4}\log\frac{88}{94-88}\right]$
8	93	·4921 $\left[=\frac{1}{8-4}\log\frac{93}{94-93}\right]$

Mean value of k=·4271

Let us now test this value of k from figure 66 which graphically represents the growth of the seedling.

(1) For instance, what will be the value of x, i.e. the growth of the seedling at the end of $2\frac{1}{2}$ days?

$$\text{Log } \frac{x}{94-x} = \cdot 427\ (t-4), \text{ where } t = 2\tfrac{1}{2}$$

$$= \cdot 427\ (-\tfrac{3}{2})$$

$$= -\cdot 64$$

$$\text{i.e.} = \bar{1}\cdot 36$$

$$= \log \cdot 229$$

$$\therefore \quad \frac{x}{94-x} = \cdot 229 \text{ or } x = \frac{94 \times \cdot 229}{1 \cdot 229} \text{ or } x = 17 \cdot 5$$

(2) Similarly the value of x at the end of $5\frac{1}{2}$ days is as follows:

$$\text{Log } \frac{x}{94-x} = \cdot 427\ (5\tfrac{1}{2}-4)$$

$$= \cdot 641 \qquad \therefore \quad x = 76 \cdot 5.$$

(3) Similarly the value of x at the end of $7\frac{1}{2}$ days is as follows:

$$\text{Log } \frac{x}{94-x} = \cdot 427\ (7\tfrac{1}{2}-4)$$

$$= 1 \cdot 494$$

$$\therefore \quad x = 91 \cdot 08.$$

These calculated growth values at $2\frac{1}{2}$, $5\frac{1}{2}$ and $7\frac{1}{2}$ days are shown in Figure 67 by small cross marks.

They are close approximations to the actual growth curve.

But it is not always correct to compare the growth rate of a plant of one species with another, because the length of growing zones differ in different plants.

For instance, in the case of certain aerial roots the length of a growing zone may be as great as 1 meter. For roots in general it may be from 5—10 mm. Again in bamboo, growing zones are several centimeters while in *Botrytis* it is only about ·20 mm. But bamboo shows only twice the growth rate of *Botrytis* if calculated as elongation per minute as percentage of growing zone.

The *true rate of growth* is defined as the growth of a unit of length in a unit of time.

Measurement of Growth

Several types of apparatus are available for measuring growth viz., (*a*) horizontal microscopes, (*b*) auxographs and auxonometers, (*c*) crescographs, etc.

(*a*) The method of measuring growth by the horizontal microscope consists in focussing the microscope (Fig. 68) upon the growing organ such as a root tip, previously marked with India ink. These marks are made at definite distances and the increase in the distance can easily be read off by the microscope.

(*b*) Auxographs and auxonometers usually give an enlargement of 20 to 40 times and are of various designs. The simplest auxonometer shown in Figure 69 consists of a long counter-balanced pointer the free end of which moves along a large graduated arc. At the fulcrum there is a pulley over which a thread previously attached to the tip of a potted plant passes and a weight is attached to the free end. As the plant elongates the magnified growth increments are read directly on the

graduated arc. The self-recording auxograph is more or less similar in design, but has a number of pulleys

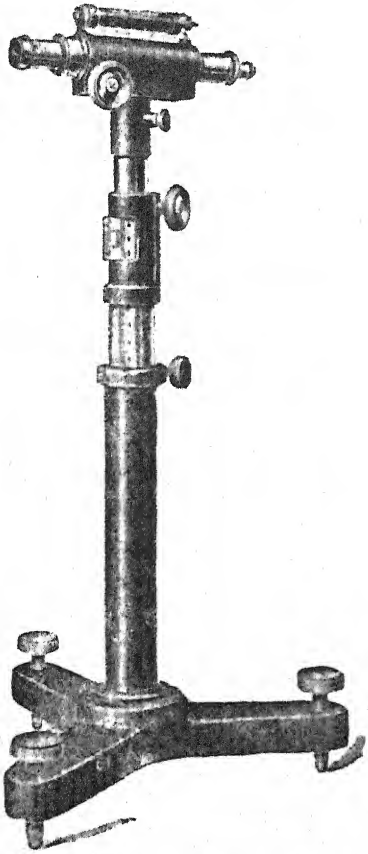


Fig. 68.—Horizontal microscope.

and wheels to magnify the growth rate which is recorded on the surface of a revolving drum covered with smoked paper.

(*c*) The elongation of a plant ordinarily is very slow being about 1/100000 inch per second.

Thus even with highly sensitive auxonometers quick changes in growth rates cannot be detected. Bose has

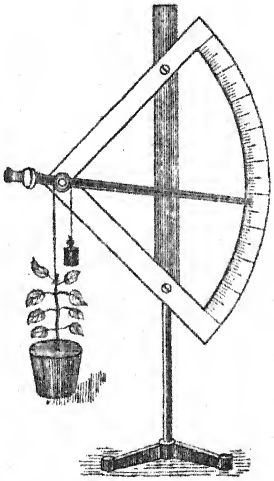


Fig. 69.—Diagram of a simple auxonometer.

devised several types of highly sensitive crescographs which can magnify anything upto 10,000 times. This he secured by means of a compound system of two or more levers; while the friction of the lever on the revolving smoked drum was removed by using a smoked glass plate which was made to oscillate to and fro, the oscillations being at regular intervals. For demonstration purposes before a large audience he also devised a magnetic crescograph which can magnify anything up to 50 million times.

But however fine these instruments of measurements may be, they disregard growth in thickness, which is not necessarily proportional to the growth

in length. Moreover, plants grow faster in darkness than in light although they are loosing matter instead of gaining. Thus false ideas of growth are arrived at when growth rate is measured in light. A better system is to measure the rate at which the plants increase their solid material viz., to measure the increase in dry weight. This has, however, one great disadvantage that the plant has to be killed so that only one determination can be taken from one individual. Thus for successive determinations a number of plants have to be killed which will undoubtedly lead to slight errors due to individual variations. But this method inspite of the above disadvantage has been found to be very useful and has been applied to a greater extent in recent works.

Conditioning Factors

As in the case of anabolic and katabolic processes there are certain external and internal factors that govern the rate, so also there are conditioning factors that govern the rate of growth of plants. The following are some of the important external and internal conditioning factors.

External—1. Temperature
2. Light
3. Water
4. Nutrition

Internal—1. Determinants. (Certain determinants are inherited on Mendelian lines which causes the offspring to be like the parents. A discussion of this is beyond the scope of this book).

TEMPERATURE.

With the increase of temperature the rates of both carbon assimilation and respiration are augmented but ordinarily assimilation far exceeds the respiration rate and food is stored. But increased oxidation also brings about an increased activity of the meristematic cells causing rapid conversion of the stored materials into the living protoplasm. As protoplasm increases rapid divisions follow. Very high temperatures are of course injurious and bring about cessation of all growth. This is supposed to be due to the accumulation of harmful substances produced as a result of high katabolism. So that up to a certain limit enhanced temperature increases the growth rate which follows the Vant Hoff's law of Q_{10}, i.e. for a rise of 10°C. the rate of growth is nearly doubled. At high temperatures, various parts of the plant are effected in a complex way and retardation of growth sets in.

Leitch (1916) working on pea seedlings concludes that there is a uniform increase of growth from −2 °C. to 29 °C. Beyond this there is much fluctuation. At 30 °C. and 35 °C. the enhanced growth rate remains only for 10 min. After this short period of enhanced activity, the growth rate steadily falls for half an hour.

This is followed by a second increase showing slight recovery. But again after a brief period of 1 to 2

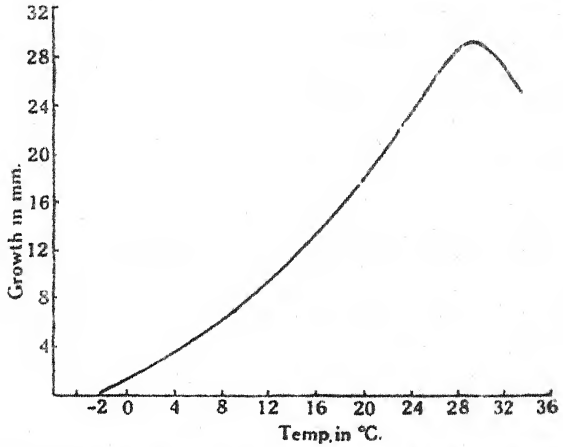


Fig. 70.—Diagram illustrating growth at different temperatures (after Leitch).

hours a steady but gradual fall, hour after hour, is noticed. From her experiments Leitch suggests the following main points.

That there is a 1. Minimum temperature. The lowest temperature at which growth will occur.

2. Maximum temperature. The highest temperature at which growth will take place.

3. Optimum temperature. The highest temperature at which time factor is not involved.

4. Maximum rate temperature. The temperature at which the rate is highest.

The minimum temperature at which growth can go on may be lower than 0 °C. as found for plants growing in temperate regions, while plants of tropical countries may stop their growth below 10 °C. Moreover plants growing in different seasons behave differently towards temperatures. In the United Provinces winter annuals show growth even at 2 C. and if they are grown in the monsoon season they show signs of 'forcing' and are weaker and in many cases never flower.

Dormancy.

In the cases of certain seeds, germination will not take place at once even though external conditions may be very favourable. These seeds undergo a period of *dormancy*. This may be due to the incomplete development of the embryo, which goes on developing after the seed is shed on the ground. In other cases the seeds may apparently be fully developed, yet certain physiological changes go on, such as the formation of the enzymes, etc. which delay development. Lastly environmental factors may effect germination *e.g.*, temperature. Temperature not only effects the germination of seeds, but buds and branches of many trees undergo dormancy in winter. In this condition respiration goes on though at a very slow rate. Such dormant buds, if immersed in water at 30 °C. for 9 to 12 hours, will again show vigorous growth. The effect, however, of such a treatment is purely local and it has been seen that while the treated branch on one side of a tree may

show vigorous growth the untreated side remains dormant.

Various methods have been used to overcome dormancy of different plant organs such as stems, tubers and chiefly the seeds. Raising for lowering the temperature, as mentioned above, or desiccation for breaking dormancy have been used in the cases of stems and branches. Solution of chemicals like acetylene tetrachloride, ethylene chlorohydrin, ether, chloroform have been very widely used for all types of plant organs for breaking dormancy. In numerous cases ethylene and hydrogen sulphide gases have been employed with success. Much work has been done regarding overcoming the dormancy of potato tubers. Appleman (1918) has noted that the buds (so called "eyes") of potato, open three to six weeks earlier than otherwise when treated with suitable concentration of ether or chloroform.

LIGHT.

Unlike suitable temperatures light is not such a necessary factor for plant growth. In fact certain lower organisms complete their life cycle in the total absence of light. On the contrary, higher plants need light for the balanced functioning of their metabolic activities. In the total absence of light the stem becomes elongated, the leaf blades become thin and loose their chlorophyll. Such plants are *etiolated.*

For the study of etiolated plants potato affords the best example as it contains considerable amount of

reserve food material for prolonged growth in total darkness. The stem as shown in figure 72 becomes

Fig. 71.—Normal potato plant.

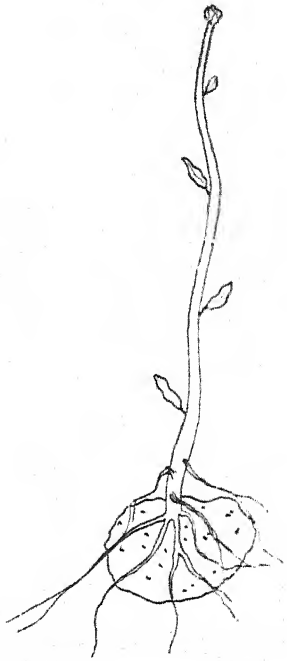


Fig. 72.—Etiolated potato plant.

elongated and the leaves are thin and devoid of chlorophyll. A transverse section of the lamina (Fig. 73) shows poorly developed palisade parenchyma as also fewer chloroplasts. A transverse section of the stem (Fig. 74) shows poorly developed vascular bundles as also the absence of strengthening tissues.

These internal changes in the total absence of light are more fundamental than a mere inertia of the

chemical reactions as evinced by the lowering of the temperature. Some people thus ascribe the important

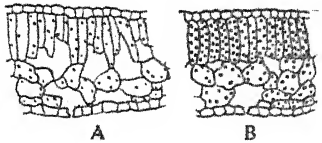


Fig. 73.—Cross section of leaves from etiolated (A) and normal (B) potato plants.

role of hormone producer to light. This idea is further strengthened by the fact that in certain cases daily

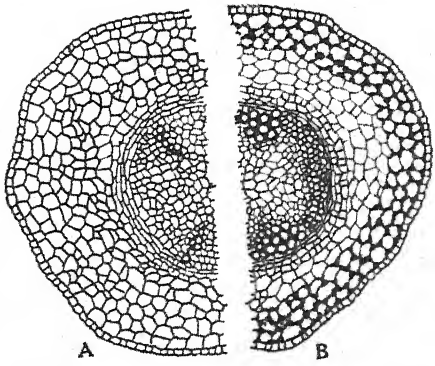


Fig. 74.—Cross section of stems from etiolated (A) and normal (B) potato plants.

exposures for only 5 minutes have been sufficient to produce normal healthy plants. A point of special interest in this connexion is that those rays that produce the maximum effect upon the photosynthetic rate have the least effect upon growth. Thus red light which

has great influence on photosynthesis has no effect upon the growth rate and plants exposed to this show the same behaviour as if they were in darkness. On the contrary violet and ultraviolet light has a great retarding influence upon growth.

In this connexion Blaauw has performed a number of interesting experiments. For his experimental materials he took such widely divergent plant tissues as sporangiophores of *Pilobolus*, hypocotyls of *Helianthus* and mustard roots. He measured the growth rate in dull red light through a horizontal microscope after experimenting with both flash light and continuous light. In the case of the sporangiophores of *Pilobolus*, in flash light, there was an induction period varying from 3 to 5 minutes according to the intensity of light supplied. In this period the rate of growth continued to be the same as in darkness. Then followed the *primary growth reaction*. Here the rate of growth rapidly increased to a maximum in about 8 minutes after which in the *secondary growth reaction*, the rate fell to below the rate of growth in darkness. Normally the time taken was about 10 minutes. This was followed by *oscillatory subsidence*, which lasted for about 20 minutes. Thus in about 40 minutes after receiving the flash light stimulus, the plant assumed the normal growth rate in darkness.

In continuous light the response by the plant was very similar to that found with the flash light, the only difference being that the final growth rate, after the oscillatory subsidence was enhanced. In the case of

the hypocotyl of *Helianthus* the response was reversed. In flash light, after a brief induction period the primary growth reaction was negative followed by a positive secondary growth reaction. There was then the usual oscillatory subsidence when the plant assumed the usual growth in darkness. In continuous light, however, the final growth rate as contrasted with *Pilobolus* was less.

Blaauw found that except the mustard root all other roots were insensible to light. The response of mustard root is shown to be very similar to that of the hypocotyl of *Helianthus*.

WATER.

Growth is always accompanied by accumulation of water. So its intake should be greater than the loss to secure growth. As water is taken in by the plant from the soil and is lost through transpiration any changes in soil moisture will affect the growth rate. Thus the water condition of the plant can be altered by any one or all of the following conditions.

(1) Change in the power of the soil to supply water,

(2) Change in the power of the roots to take up water, and

(3) Change in the power of the air to remove water.

So that when the $\frac{\text{intake}}{\text{outgo}}$ is less than unity there is no growth. There are some forms of plants that grow

well in dry climate while others in moist climate. Plants thriving in dry climates have structures that facilitate absorption and hinder transpiration. In relatively humid conditions the internodes are generally long, the cuticle thin and woody tissues less developed. The reverse is found in arid conditions. The latter promote thorns and spines or may cause succulence. A familiar example of the effect of humid and arid conditions on the same plant is furnished by *Tropaeolum majus*. This plant when grown in moist conditions forms leaves 5 times as large as those in dry conditions and the cuticle remains thin. While no collenchyma is formed in moist conditions there is a tendency towards formation of a well developed collenchyma in dry conditions.

Nutrition.

The presence of nutritive elements e.g., carbon, hydrogen, oxygen, nitrogen, sulphur, and phosphorus are absolutely essential for growth as these are the substances that go to form carbohydrates, fats and proteins which ultimately lead to the formation of the living protoplasm, an increase of which alone gives the true index of growth. Growth implies increased vegetative or reproductive activities. During recent years as a result of extensive researches a wealth of literature has cropped up regarding the factors that influence either the vegetative or the reproductive phases.

Some of the important works in this connection are outlined under photoperiodism.

Photoperiodism.

Although it had been known for a long time that the duration of exposure to light affects growth, Garner and Allard (1920) were first to critically analyse the effect of light on plant growth. They suggested the term *photoperiodism* to designate the response of organisms to the relative length of day and night and *photoperiod* to designate the favourable length of day for each organism. Garner and Allard have accordingly grouped the plants in their behaviour towards the length of light exposure to three types.

(*a*) Those plants whose times of flowering are unaffected by the duration of light exposure. Only a few plants fall under this category.

(*b*) Plants which flower by the action of short day. Such plants are called *short day* plants. The majority of the plants belong to this category. Some of the examples are *Phaseolus vulgaris, Chrysanthemum, Aster linariifolious* etc.

(*c*) Those plants which flower as a result of long exposure to light. These have been designated as *long day* plants. Only some plants such as *Hibiscus*, radish, perennial rye grass come under this head.

Kraus and Kraybill were amongst the first to emphasise that much of the differences in flowering of the short day and long day plants depends upon the carbohydrate nitrogen (C/N) balance in them. Working on tomato plants they come to the following conclusions:—

(1) With a very high carbohydrate/nitrogen ratio on account of a small amount of nitrogen and high carbohydrate content there is weak vegetative growth and the plants are non-fruitful. Here nitrogen seems to be the limiting factor for growth.

(2) With a high carbohydrate/nitrogen ratio on account of excess of carbohydrates and some available nitrogen there is lessened vegetative growth but abundant flowering and fruit production.

(3) With a low carbohydrate/nitrogen ratio on account of excess of both carbohydrates and nitrogen there is much vegetative growth but no fruit formation.

(4) With a low carbohydrate/nitrogen ratio on account of small amount of carbohydrates but excess of nitrogen there is suppression of both vegetation and fruitfulness. Here carbohydrates become limiting.

So the presence of both carbohydrates and nitrogen in a balanced proportion is essential for proper vegetative growth and fruitfulness. This proper balance of carbohydrates and nitrogen i.e. C/N ratio is achieved with shorter exposure in "Short day" plants and with longer exposure to light in "Long day" plants.

Nightingale (1927) and Eckerson (1932) have suggested that the balance between carbohydrates and nitrogen is influenced by the length of exposure to light because the latter affects the production of elaborated nitrogen in the plants. For the "Short day" plants short periods of daily exposure are sufficient for adequate assimilated nitrogen which with excess of carbo-

hydrates results in fruit formation. With longer exposures these short day plants become richer in nitrogen, C/N ratio gets altered and consequently there is much vegetative growth and fruitlessness. Similarly long day plants get their proper amount of elaborated nitrogen with longer exposures to light. With short day conditions they have less elaborated nitrogen and have poorer vegetation and fruitlessness. If this period of exposure is increased they manufacture more elaborated nitrogen, proper C/N ratio is reached and vegetation and fruitfulness appear.

Recent work has shown however that to produce the same effects on different plants, the same carbohydrate/nitrogen ratio may not hold good. Thus in one case the carbohydrate/nitrogen ratio may be from 4 to 6 to produce fructification while for another plant of a different family it may be from 8 to 12.

An explanation of photoperiodism is furnished by the work of Lysenko on the vernalization of wheat. According to Lysenko's theory of Phasic development of annual seed crops there are several phases in the development cycle of which three phases have so far been clearly identified. The period from germination to inflorescence are included in the first and second phase, and the third phase covers the period of production of gametes. The requirements of the first phase is specific temperature, of the second phase temperature and photoperiod, and of the third phase photoperiod only. Accordingly the concept of photoperiodism can be included in his theory of phasic development. (For fur-

ther details the reader is requested to read the Chapter on Vernalization).

Vernalization

This term is used to denote the practical agricultural method of accelerating the development of plants. In colder countries like Europe, there are cereals of two physiological kinds viz., the winter cereals and the spring cereals. Taking wheat for example, the winter varieties are sown in September or October. They come out about 6″ from the ground and then remain dormant through the winter months. During spring they again burst forth into activity. The spring varieties of wheat are sown after the winter and they flower and fruit the same year. On the other hand when winter wheats are sown in spring the conditions are unfavourable for the completion of certain stages of their development, which are thus inhibited, and the plants appear to remain for an indefinite period at the stage of tillering i.e., their growth continues but their development is arrested. Lysenko, the Russian physiologist, has however evolved a method by which he is able to transform the winter wheats into spring ones i.e., when winter wheats are sown even in spring flowering takes place.

His theoretical conceptions are as follows:—

(1) Growth and development are not identical phenomenon.

(2) The entire process of development of an annual seed plant consists of individual stages.

(3) The stages always proceed in a strict sequence and a subsequent stage cannot set in until the preceding stage has been completed.

(4) Different stages of development of the same plant require for their completion different external conditions.

The technique of vernalization consists in soaking the seeds in water, which transfers it from a state of rest into a state of active life. But soaking is not continued until complete absorption of water, and growth is thus prevented. In the case of the transformation of the winter plants into spring ones, the principal factor is the influence of low temperature. These partially soaked and therefore slowly germinating seeds of winter plants are exposed to a temperature of about 0° C. for a period ranging from 15 to 60 days, after which they acquire the properties of spring plants. If they are sown in spring they will ear the same summer. This remarkable behaviour is due to the fact that growth and development are not the same phenomenon. Growth is an increase in the size of a plant without any profound qualitative changes in the growing parts. While in development the plant enters a new stage, qualitatively differing from the preceding stage and bringing it nearer to its final phase of life, viz., fruit bearing.

Thus the cause of unlimited tillering when winter wheat is sown in spring, is the adverse temperature which accelerates growth but not development. On the contrary, in a slightly sprouting wheat seed, under

an appropriate complex of external conditions, development may proceed so much that the flowering may take place even though it is sown in spring. Unlike wheat, there are plants that need increased temperatures as a factor of vernalization e.g., millet, bean, and cotton, The germinating seeds of these must be exposed for several days to a temperature of 20° to 30° C.; after this they pass rapidly to the stage of fruit bearing even in cool climates.

Practical Experiments

1. *Experiment to show distribution of growth regions in root.*—The roots of the *Narcissus* plant are marked at equal distances. The bulb of the plant is then put on the top of a bottle containing water. After some hours the distances between the marks are measured. It is noticed that the distance between the marks just below the root tip has increased much more than at any other region.

2. *To measure growth in roots by a horizontal microscope.*—The root of a germinating grain seed is introduced into a thistle funnel which dips in a beaker containing water. The root is marked at equal distances and the marks are focussed through the microscope. As growth takes place the distance between marks increases, which can be measured by the microscope.

3. *The measurement of growth by an arc indicator.*—The tip of a potted plant is tied by a thread which passes round a pulley with a weight at the other end. To the pulley is attached a pointer which moves along an arc scale. As growth takes place the weight comes down and the pointer moves over the scale (see Fig. 69).

4. *Experiment to show that oxygen is necessary for growth.*—A long tube containing mercury is inverted

over a dish of mercury. Some hydrogen gas is introduced into the tube. Some seeds are allowed to grow in the hydrogen gas. It is noticed that no growth takes place, showing that oxygen is necessary for growth.

5. *Experiment to show that both moisture and oxygen are necessary for growth.*—Three germinating pea seedlings are fixed with pins at different levels on a flat piece of wood immersed in water in such a way that one of the seedlings is completely inside water, another is just at the level of water, while the third one is in air above the level of water. After a day or two it is noticed that the seedling in air dies for want of moisture and the one inside water also dies for want of oxygen required for respiration, while the third one at the level of water continues to live as it is supplied with both water and air.

6. *Experiment.*—Draw coleoptiles of Barley, emphasizing the region of curvature.

CHAPTER X

MOVEMENTS IN PLANTS

The External and Internal Stimuli

Many people believe that plants lack the capacity to move, because they are fixed firmly by the roots to the ground. This idea is, however, erroneous; for movements take place in plants though slowly, as for example, the orientation of leaves in the sun, the movements of the chloroplasts etc.

Unlike animals, plants have simple mechanisms for movement: but like animals, these movements are affected by definite external and internal stimuli.

To bring about any movement (1) a stimulus must be perceived, (2) it must cause a definite state of excitation at the region of perception, and (3) transmission of the stimulus must occur if the regions of perception and movement are far apart. This is analogous to the reflex movement in animals. In the latter case, however, they have muscles and nerves. Plants have none of these. This does not mean that plants as a whole are lowly evolved. The slow rate of movement and the low degree of perception and transmission are indicative of a specialised type of evolution.

The lower plants like *Chlamydomonas* have as rapid and efficient organs of locomotion as animals of

that degree of development e.g., Infusoria and the Flagellata. As plants evolved to higher forms, the characteristic mode of nutrition did away with the necessity of rapid locomotion and the consequent development of the complex organs of locomotion.

The Energy Involved in Movements.—The energy of stimulus has no relation to the energy of response. A little stimulus may produce a great deal of response. Thus stimulus liberates a great deal of energy which must have been already present in the plant. The reaction may be likened to the action of striking a match by which a small initial friction releases a very large amount of energy. In this case, however, the amount of energy obtained is not increased by striking the match more violently. Thus only a certain minimum force is necessary, anything in excess of that amount is superfluous. On the other hand, plant responses are increased or decreased, within a limited sphere, according to the strength of the stimulus.

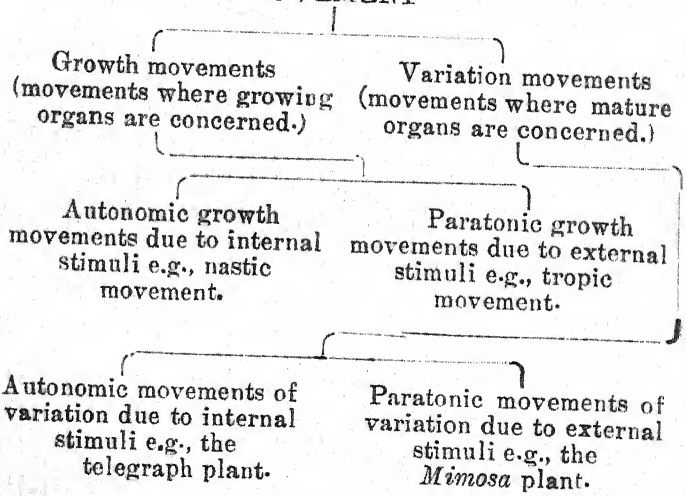
The response may be pictured thus:—

Stimulation → obscure complex vital phenomena → reaction or response, i.e., movement.

Why does a plant react in a particular way? The answer to this query is difficult and the explanation leads us beyond the experimental stage. Sir Francis Darwin believes that the past experiences of the race enables the plant to react in a definite way to a stimulus. This does not imply that the plant does it consciously or that it has a memory, as we have. He says

the individual acts by that unconscious memory which is inheritance.

All plant movements can be classified as under:—



Growth Movements

(*a*) Autonomic Growth Movements.

The rate of elongation of roots and shoots among other causes is dependent upon internal causes. It has been seen that the growth is never in a straight line but is zig-zag. This is called *nutation.*

Among other autonomic growth movements are the *nastic* movements. *Epinasty* is the increased growth on the upper side of an organ as compared with the lower. The result being the downward bending of that organ, as in the poppy flower stalk. When half-grown due to epinasty it is kept down but when the

flower is mature *hyponasty* i.e., more growth on the lower side, takes place as a result of which the stalk straightens.

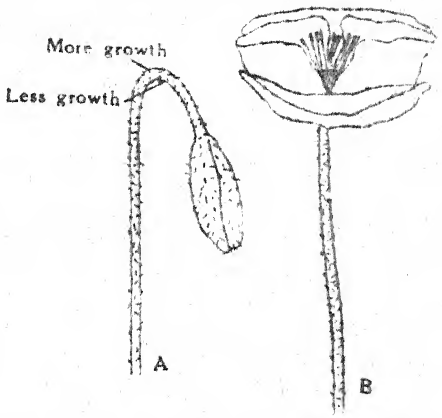


Fig. 75.—Poppy flower bud (A) showing epinasty due to more growth on the upper side and mature flower (B) with upright stalk as a result of subsequent hyponasty i.e., more growth on the lower side.

This nastic bending can be seen in the developing fern leaves also.

(*b*) PARATONIC GROWTH MOVEMENTS.

The two most important forces influencing the direction of plant growth are:—

(1) Gravity and (2) light; a third factor (3) moisture may also play a part.

GEOTROPISM.

The force of gravity is acting on all matters including plants. This pull of gravity is in one direction only to which the various plant organs respond in

diverse ways. Response by such one sided stimulation is called *tropism*. Tropism induced by 'Geo' (earth) is called *geotropism*.

If the growth of the organ is towards the centre of the earth, as in the case of roots, it is *positive geotropism;* away from it, as in stems, it is *negative geotropism;* or at right angles to this direction, as in branches, leaves etc., it is *diageotropism*.

The reaction to the stimulation of gravity can easily be shown by placing a young root at right angles to the vertical. Its tip begins to curve downwards due to more growth of cells on the upper side. A deviation of even one degree from the vertical produces a curvature.

The effect of gravity can be avoided, if plants are rotated, so as to get them stimulated on all sides equally. A machine that secures this result is the klinostat (Figure 76).

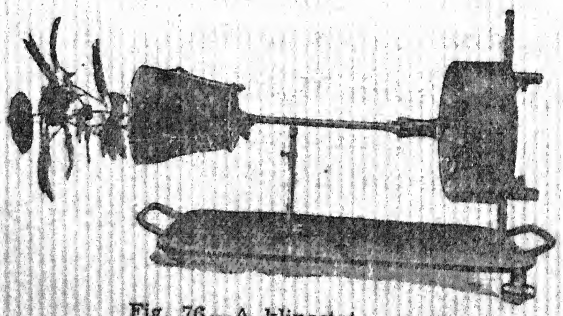


Fig. 76.—A klinostat.

If a potted plant is placed horizontally on a klinostat and is slowly rotated, the shoot will continue to grow horizontally simply because it is stimulated by

gravity equally on all sides. But during its rotation if the klinostat is made to halt for some minutes at some particular place then the shoot may show *negative geotropism*, because the cells nearest to the source of gravity are stimulated to a greater extent than the other cells.

The minimum period of exposure to the one-sided stimulus which produces curvature afterwards, while the plant rotates on the klinostat is called the *presentation time.*

It may happen that the plant may have received sufficient stimulus but its visible effect on one sided growth may take a little longer time. Thus the minimum period taken by the plant to produce a visible curve is called the *reaction time.*

Again it may so happen that a plant rotating on a klinostat may be made to stop for a very brief period after each complete revolution. This period may be so small that the plant may perceive the stimulus but may not give a visible response. The time required to perceive this minimum stimulus is called the *perception time.* But if time after time this stimulus is given at the same place before the internal stimulus of perception dies out then the accumulated effect of the stimuli may be strong enough to create a curvature or response by the plant. On the other hand if the klinostat is rotated very slowly so that the first stimulus of perception dies out before the second is received then the plant will never show a response. The time taken for this stimulus to die out is the *relaxation time.*

Historically there are two main theories of geotropic response, viz., (a) *statolith theory* and (b) *hydrion differentiation theory*.

1. *Statolith theory*.—Statolith theory was propounded by Pfeffer. According to him the starch grains which are present in the protoplasmic matrix of each cell, due to gravitation, fall at the bases of the cells

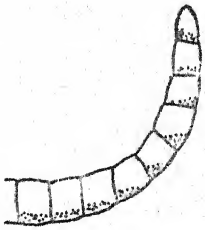


Fig. 77.—Diagram to illustrate statolith theory. The starch grains fall to the bases of the cells under gravitational force causing the cells to react.

thus creating an irritation on the sides of the horizontally lying cells, causing them to react. The reaction of the stems and the roots are however different, the former tending to react against gravitation and the latter towards it.

In many plants, however, there are no starch grains, and thus there should be no response in these cases. But the expounders of the theory have modified the original theory slightly and suggest that the falling of any granules inside the cell will constitute a stimulus.

2. *Hydrion Differentiation Theory*.—This theory is based on the fact that proteins which form a large

part of the cell protoplasm are amphoteric electrolytes. At a definite pH they are electrically neutral. This is the isoelectric point which varies between 4 to 5·5 pH. Suppose at 4·5 pH in a particular case they carry a positive charge and at 6·5 pH a negative charge. In plant cells these proteins along with lipoids cream up, much in the same way as the cream comes up in boiled milk. Due to this creaming up a difference in electric potential takes place in each cell. So that each cell really becomes a small Leclansch's cell, and if it is connected with a sensitive galvanometer an electric current may be detected. The direction of the flow of the current will depend upon whether the creamed up protein carries a positive or a negative charge which in its turn will depend upon whether the pH is relatively alkaline or acid.

The pH of stems are relatively alkaline because the carbonic acid formed as a result of respiration quickly escapes out in the atmosphere, through efficient respiratory channels *i.e.*, the inter-cellular spaces. the lenticels and the stomata. On the other hand, roots are relatively acid as the carbonic acid of respiration cannot escape out so efficiently.

Thus the current moves in one direction in the stem and in the opposite direction in roots.

When a primary root is placed horizontally as shown in figure 78 an electric current is generated which travels in the direction of the arrows. But during the passage of the current through cell to cell, due to resistances much of the electricity leaks out, so that by the

time the current re-enters the cells generating the electricity it becomes very feeble. It is well known that a

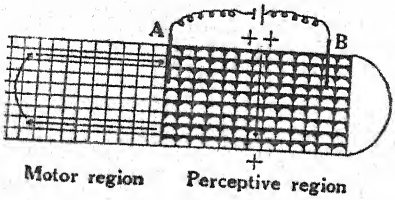


Fig. 78.—Diagram of root apex to illustrate hydrion differentiation theory. The creaming is indicated in the perceptive region; arrows indicate the path of current. As current moves from less positive to more positive, more current passes on the lower side of root through the motor region; but as it moves, due to high resistances, it continuously leaks out, so that less current passes through the upper. Thus the cells of the lower region are more permeable and show less growth, while on the upper region the cells are less permeable and show more growth resulting in a downward curvature of the root.

strong current increases the permeability and thus retards growth. Thus in the case of a horizontally placed root there will be less growth towards the lower side and the tip will bend down.

In the stem on the other hand the current will flow in the opposite direction resulting in less growth on the upper side and so the stem tip will curve up.

Growth Regulators.—As far back as the middle of the nineteenth century Charles Darwin showed in the cases of coleoptiles of various grasses that the region of perception and the region of curvature were not the same. In recent years this work has been confirmed again and again by various investigators. In the case of the coleoptiles of grasses the curvature takes place at the bases of the excited tips. Thus there is a perceptive

region and a responsive region. It follows, then, that the excitation is generated at the tip which travels down and brings about the curvature. That there is no nerve mechanism involved in this is shown by the following experiments.

When the roots of *Vicia faba* were excised at a distance of 2 mm. from the tips and subsequently geotropically stimulated, no response took place. But when the tips were replaced by sticking them with gelatin to the cut ends curvature was shown. Curvature was also shown to take place when the tip was simply replaced with a thin film of water. On the other hand, when a thin piece of mica was inserted between the tip and the cut end of the root no curvature occurred. Thus it clearly shows that some substance evidently a growth promoting one (see page 180), is produced at the tip as a result of geotropic stimulation which travels down to the responsive region. The most peculiar feature of this substance is that it causes the stem to react one way and the root the other way. If an excised tip of a stem is fixed with gelatin to the root whose tip has been previously removed the root will show positive geotropism. This shows that the growth regulating substance is the same for both the stem tip and the root tip.

Phototropism.

The capacity of turning towards or away from light is *phototropism,* or to use the older word *heliotropism* from Helios which means sun. Like geotropic

responses, various plants and plant organs respond variously to one sided illumination, and the responses are classified accordingly.

Positive phototropism is shown by coleoptiles of grass and primary shoots. Here the plant organs move towards the source of light. *Negative phototropism* is shown by some primary roots *e.g.*, mustard, and is the movement of the root away from the source of light. Most roots, are, however, insensible to the action of light.

Diaphototropism is shown by leaves; it is the orientation of the leaves so as to be perpendicular to the source of light..

In plant parts of the category of hypocotyls of Sunflower which shows negative primary light growth reaction (refer page 202) there is positive phototropic movement because the cells nearest to the source of light grow less than the cells away from the source, resulting in the curvature of the shoot towards the source of light. Negative phototropic reaction in some cases takes place because there the cells act as concentrating lenses whereby the distal part gets stronger light and shows retarted growth on that side resulting in the bending of the shoot away from the source of light. (See Fig. 79).

In the second category is included the sporangiophore of *Pilobolus* which has a positive primary light growth reaction. This should then bend away from the source of light on the analogy of the hypocotyl of Helianthus. But in this case also the bending takes

place towards the source of light. This is due to the sporangiophore acting as a lens whereby light is concentrated on the wall away from the source of light. There is, therefore, more growth on the distal side resulting in positive bend.

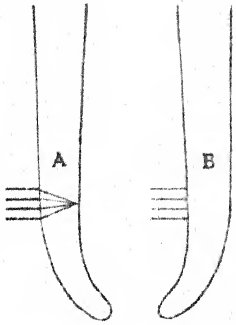


Fig. 79.—Diagram illustrating the positive and negative phototropic reactions in tissues having negative light growth reaction. Note that in (A) the cells act like lenses and the rays converge, illuminating the distal end more strongly; thus the organ bends away from the source of light.

As in the case of geotropic response, here too, the region of perception and the region of response are different. It has been shown in the case of seedlings of certain cereals that when they are kept in a phototropic chamber the hypocotyl will form a distinct curve and the tip of the plumule will be directed towards light. It has been shown, however, that the plumule alone can perceive the light stimulus while the hypocotyl responds to this stimulus. If a black paper is put to cover the plumule and the hypocotyl is exposed to light no curvature will take place. But if hypocotyl

is covered and the plumule is exposed then the curvature of hypocotyl will take place.

Thus a leaf blade perceives a stimulus but the petiole responds to it. It is possible that a unilateral exposure to light causes certain inhibitory substances to appear in the organ of perception which descends to the growing zone and produce a retardation of growth on the exposed side.

As has been shown in the case of geotropism, these growth regulators are definite substances which diffuse to lower organs not only through living cells but also through such substances as gelatin and water. If mica or tin is placed instead of gelatin then there is no transmission of stimulus. So that here it is not a case of transmission of electric charge but of diffusion of chemical substance.

However, electric polarisation of the cells takes place by unilateral illumination. The illuminated part receives the negative and the shaded the positive charge. Thus the current of growth promoting substances is shifted towards the shaded side.

Hydrotropism.

Movement or curvature of a plant organ towards water particles is referred to as *hydrotropism*. This can be demonstrated easily with the germinating seedlings of pea. Pea seeds soaked in water the previous night is kept on a wire gauze covered with moist saw dust. The wire gauze is then kept slanting in a humid

condition. After a few days the radicles will be seen bending towards the moist saw dust (Fig. 80).

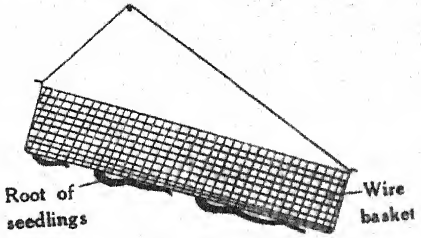


Fig. 80.—Experiment showing the hydrotropism of roots.

Chemotropism is the movement of plant towards a chemical substance *e.g.*, the growth of pollen tubes towards an ovule which secretes a sugary substance.

Movements of Variation

(*a*) Autonomic Movements of Variation.

A very fine example of movement under this head is afforded by the telegraph plant *Desmodium gyrans*. Given normal external conditions the lateral leaflets of this plant shows a continuous rotatory movement in an ellipsoidal orbit. Very little detail is known concerning this type of movement.

(*b*) Paratonic Movements of Variation.

The well known example of this movement is afforded by the sensitive plant, *Mimosa pudica*. It is commonly found on the lower altitudes of the Himalayas and often grown on the plains as potted ornamental plants.

It is a herbaceous plant with compound leaves and at the base of each leaflet there is a small swollen pulvinus. A long rachis joins the leaf to the stem at whose junction also there is a large pulvinus.

When the stimulus is applied to a leaf tip by striking it with the fingers the three stages of stimulation, conduction and response are clearly illustrated. The leaflets nearest to the affected tip first close in pairs. The stimulation is next conducted to other leaflets which go on closing in pairs one after another. In the meantime, the main leaf stalk drops, the pulvinus at its base acting as a hinge.

If the stimulation is sufficiently strong, it is conducted along other axes where it starts from the bottom and works upwards, as the stimulating effect is conducted.

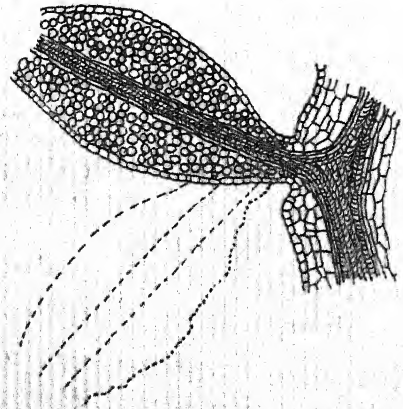


Fig. 81.—Longitudinal section of a pulvinus of leaf of *Mimosa* attached to the stem.

As has been said before the pulvini in each case act as hinges causing a visible response to the stimulus.

Figure 81 shows a sketch of longitudinal section through a pulvinus.

The vascular bundle passing through the pulvinus roughly divides it into an upper stable and a lower sensitive half. The lower part has also delicate hairs. If the hairs are touched roughly with the fingers, then the sap from cells of the lower half of the pulvinus gets injected into the inter-cellular spaces causing these cells to loose their turgor. The normal position of the leaf stalk is a resultant of the osmotic forces of the upper and the lower halves of the pulvines. So that when the lower half looses its turgidity its power of upward thrust is lost and consequently the leaf stalk drops.

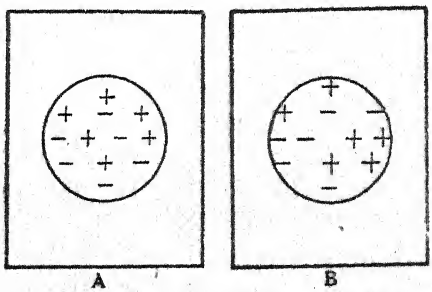


Fig. 82.—Diagram to illustrate the impingement of ions on the cytoplasm lining the vacuole (B) where they are withheld when excited; A, unexcited cell.

The causes for this loss of turgor of the cells of the lower half of the pulvinus is not fully known but it is now believed to be due to a hormone which excites the cytoplasm. The excited cells then withhold the osmotically active ions of the sap when they impinge

on the protoplasm during their kinetic flight. Thus when these solutes are withdrawn, the osmotic pressure falls and the water gets ejected. During the slow recovery, the solutes are gradually released from the cytoplasm, causing a slow increase of the osmotic pressure and the consequent withdrawl of water from the intercelullar spaces.

Practical Experiments

1. *Phototropism.*—A potted plant is put in a box which has a hole on one side. After a few days it is observed that the growing tip with the younger leaves bends towards the hole through which light is coming.

2. *Geotropism.*—A potted plant is put in a horizontal position. After a day its growing tip is found to bend upwards showing that the stem is negatively geotropic.

3. *Hydrotropism.*—Some seeds are germinated in moist soil on a wire gauze (Fig. 80). It is seen that the roots grow downwards due to positive geotropism, but later they are seen again to bend up towards the moist soil. This shows that roots are positively hydrotropic.

4. *Experiment to show that roots are positively geotropic and negatively heliotropic.*—In a glass box is put some moist sawdust and seeds are germinated there. The box is covered on three sides by a black cloth. It is seen that the roots bend inwards i.e., towards darkness and downwards.

5. *Klinostat: (a) Horizontal.*—A potted plant is fixed to a horizontal klinostat which on account of the clockwork rotates on its own axis. If the instrument is not worked the stem will bend upwards and the roots

will go downwards. But if it be slowly rotated then the plant will continue to grow horizontally, because each side of the axis is in turn directed downwards. On very rapid revolution, however, a centrifugal force will be developed and the roots will bend obliquely outwards and the stem obliquely inwards.

(*b*) *Vertical.*—A few seedlings are attached to the cork of the vertical klinostat. The growth will be normal if the machine is not moving. If the cork rotates so that the centrifugal force and the force of gravity are equal, then the roots diverge to an angle of 45° and the stems converge at an angle of 45°. But if the wheel is rotated more rapidly, the centrifugal force will be greater at the periphery and roots will become quite horizontal.

APPENDIX A

LOGARITHMS

By the help of logarithmic method much of the complicated multiplications and divisions in arithmetic can be simplified.

The meaning of indices.—The expression a^m simply means the product of m factors each of which is equal to a. The letter m is called the *index* of the power to which a must be raised; and a is, therefore, called the *fundamental number* or *base*,

e.g., $10^3 = 10 \times 10 \times 10$ (*i.e.*, 3 factors); here 3 is the index and 10 is the base.

Laws of indices.—The following laws are universally true:

(1) $a^m \times a^n = a^{m+n}$ (*e.g.*, $10^4 \times 10^3 = 10^7 = 10{,}000{,}000$);

(2) $a^m \div a^n = a^{m-n}$ (*e.g.*, $10^4 \div 10^2 = 10^2 = 100$);

(3) $(a^m)^n = a^{mn}$ (*e.g.*, $(10^2)^3 = 10^6 = 1{,}000{,}000$);

(4) $(a^m)^{1/n} = a^{m/n}$ (*e.g.*, $(1000^2)^{\frac{1}{3}} = 1000^{\frac{2}{3}} = 100$);

where m and n may be positive, negative, integral or fractional.

$a^{m/n}$ means that a is to be raised to the mth power and then to have the nth root extracted.

E.g., $10^{\frac{2}{3}} = \sqrt[3]{10^2} = \sqrt[3]{100} = 5{\cdot}623$

The meaning of a zero index.—

$$\text{Since } \frac{a^m}{a^n} = a^{m-n}$$

$$\therefore \frac{a^m}{a^m} = a^{m-m} = a^0$$

$$\text{But } \frac{a^m}{a^m} = 1 \qquad \therefore a^0 = 1$$

i.e., any number raised to the power zero is equal to 1.

Logarithms:

If a be any number and x and n two other numbers such that $a^x = n$, then x is called the logarithm of n to the base a and is written $\log_a n = x$.

The log of a number to a given base is, therefore, the index of the power to which the base must be raised that it may be equal to the given number;

$$10^2 = 100 \qquad \therefore 2 = \log_{10} 100.$$

Common logarithms.—For ordinary calculation purposes logarithms are used to the base 10. Thus, if $m = \log_{10} a$, it is simply written as $m = \log a$, the base 10 being understood. Such logarithms are called *common* logarithms.

Napierian logarithms—While ordinarily logarithms are used to the base 10, it is sometimes convenient to use it to the base e, where $e = 2{\cdot}71828 \ldots$. This system of logarithm is called the Napierian system. It can be easily converted to the common logarithms. The

formula for reduction is $\log_a n = \frac{\log_b n}{\log_b a}$ or $\log_b n . \log_a b$. Thus $\log_e a = \log_{10} a . \log_e 10 = \log_{10} a \times 2{\cdot}3026$.

Antilogarithm.—By antilogarithm is meant the number corresponding to a given logarithm. Thus, if $\log 2 = {\cdot}30103$, then antilog of ·30103 is 2.

Advantages of logarithms.—

1. The log of the product of two quantities is equal to the sum of the log of the quantities to the same base; *i.e.*, $\log_a (mn) = \log_a m + \log_a n$

Let $x = \log_a m$ so that $a^x = m$,

and $y = \log_a n$ so that $a^y = n$;

then $mn = a^x \times a^y = a^{x+y}$

$\therefore \quad \log_a mn = x + y = \log_a m + \log_a n$

2. The log of the quotient of two quantities is equal to the difference of the log of the quantities to the same base; *i.e.*, $\log_a (m/n) = \log_a m - \log_a n$

Let $x = \log_a m$ so that $a^x = m$

and $y = \log_a n$ so that $a^y = n$

then, $\frac{m}{n} = \frac{a^x}{a^y} = a^{x-y}$.

$\therefore \quad \log_a (m/n) = x - y = \log_a m - \log_a n$

3. $\log a^n = n \log_a$ (For proof, see any text book of trigonometry.)

With the help of these formulae and the log tables complicated multiplications, divisions, extraction of roots etc., can be much simplified.

APPENDIX B

H-ION CONCENTRATION

A normal solution of any acid is defined as one containing *one gram of Hydrogen* or its equivalent dissolved in *one litre* of water. Accordingly the weights of HCl, HNO_3 and CH_3COOH or any other monobasic acid contained in 1 litre of normal acid would be the respective molecular weights in grams.

$HCl = 36{\cdot}5$, $HNO_3 = 63$; $CH_3COOH = 60$

In he case of dibasic or tribasic acid, it will be the molecular weight divided by 2 or 3 respectively.

$$\frac{H_2SO_4}{2} = 49 ; \quad \frac{H_3PO_4}{3} = 32{\cdot}6$$

Thus while the normal solutions of all these acids contain in the litre different quantities of the acid, they all contain the same quantity of H namely 1 gm. in 1 litre.

Again though they contain the same amount of H, it does not follow that the whole of this amount is ionized. The actual acidity of a solution is measured by the proportion of ionized H atoms it contains, and therefore the actual acidity of equinormal solutions of the above acids may be very different. Their titratable value will of course be the same.

Now HCl which is a strong acid, is almost entirely ionized in dilute solutions, while acetic acid in equivalent strengths is ionized to a much smaller extent.

In fact about 97% of the H in a 0·001 N solution of HCl is ionized and only 84% in a Normal solution, while in 0·001 N acetic acid, not more than 13·6% of the H is ionized. Thus the two have the same normality e.g., the alkali neutralizing power is the same, but the percentage of ionized H is seven times as great in HCl as in that of CH_3COOH.

The reason why the feebly ionized CH_3COOH ultimately requires the same quantity of alkali as the strong HCl is due to the fact that as the hydrogen ions, in the acetic acid are neutralized a fresh quantity of unionized H becomes ionized to take the place of those which have been neutralized. To preserve equalibrium as illustrated below in the application of Law of mass action.

It is the ionized H only which is responsible for the acidity of a solution and so the knowledge of H-ion concentration, for biochemical purposes is of prime importance. Taking the case of water, even the purest one, dissociates to a minute extent into H^+ and OH^- ions. Thus a solution will contain a large quantity of HOH molecules and a certain concentration of H^+ and OH-ions. The relation between the undissociated molecules and its dissociated ions is governed by certain law called the "Law of Mass action", which in the above case may be expressed by the following equation.

$$\frac{(H)^+ \times (OH)^-}{HOH} = K$$

Now as HOH, as previously stated, is enormous as compared with H and OH the equation can be written thus

$$(H)^{+} \times (OH)^{-} = K$$

Careful measurements have shown K to have the value $10^{-14.14}$. And since in pure water we have an equal number of (H) and OH ions/the ionic concentration must be $(H) \times (OH) = 10^{-7.07} \times 10^{-7.07} = 10^{-14.14}$ at room temperature.

Again in a N/10 solution of HCl there would be 0·1 gm. of H in 1000 c.c. presuming it to be completely ionized. In Actual fact a N/10 solution is ionized to the extent of 91%. Consequently the concentration is only $\frac{0{\cdot}1 \times 91}{100}$, or $9{\cdot}1 \times 10^{-2}$

The concentration is more conveniently expressed as a logarithm.

$$\log_{10}{}^{9.1} = {\cdot}96$$

$$\therefore \quad 9{\cdot}1 \times 10^{-2} = 10^{{\cdot}96-2} = 10^{-1{\cdot}04}$$

It has been agreed to express H-ion concentration as the exponent to the base 10 of the concentration with the negative sign omitted and this is represented by the symbol pH. Hence the H-ion concentration of the above N/10 HCL would be

$$p\mathrm{H} = 1{\cdot}04$$

Thus at exact neutrality of water when the concentration of H and OH are equal the pH will be 7·07 (see above).

If one knows the H-ion concentrattion then there is no need to estimate the OH-ions which can easily be found from the difference between 14·14 and the H-ion concentration. Thus for pH = 2 the OH-ion concentration would be pOH=12·14.

BUFFER

In all living plants and animals provision is made that the pH is not easily disturbed. This is made possible by the presence of certain salts e.g., phosphates, or borates of the alkali metals or sodium bicarbonate. These salts exert a buffer action in counteracting any considerable increase of the pH, by the introduction of acids.

The Phosphate System.

The monosodium phosphate (NaH_2PO_4) behaves as a very weak acid; while the disodium phosphate (Na_2HPO_4) is a weak base. The dissociations of these salts takes place thus,

(1) $Na_2HPO_4 \rightleftharpoons Na^+ + Na\ HPO^-$

(2) $Na\ H_2PO_4 \rightleftharpoons Na^+ + H_2PO_4^-$

(3) $Na\ HPO_4 \rightleftharpoons Na^+ + HPO_4$

(4) $H_2PO_4 \rightleftharpoons H^+ + HPO_4$

(5) $H_2O \rightleftharpoons H^+ + OH^-$

(6) $HPO_4 + H_2O \rightleftharpoons H_2PO_4 + OH$

Thus the last two equations give an idea that the alkalinity to the solution of Na_2HPO_4 is due to the reaction in which HPO_4 combines with the H^+ of water leaving OH^- in excess.

Again, NaH_2PO_4 is an acid reaction, the source of H^+ ions is the dissociation in No. (4) above. If we combine equation 2 above we have

$$NaH_2PO_4 = Na^+ + H^+ + HPO_4^{--}$$

and in solution of

$$Na_2HPO_4 = Na^+ + Na^+ + HPO_4^{--}$$

Now if we add Na_2HPO_4 to a solution of NaH_2PO_4 an excess of HPO_4^{--} is produced. And according to the law of Mass Action we reverse the dissociation of equation (4).

$$H_2PO_4 \rightleftharpoons H^+ + HPO_4^{--}$$

so that H^+ concentration of the acid phosphate is reduced. These considerations show that phosphate mixtures very comparatively little from neutrality.

THE BICARBONATE SYSTEM.

It is highly significant that the CO_2 produced as a result of oxidations in living tissues play a great part in buffering the pH concentrations.

(1) $NaHCO_3 \rightleftharpoons Na^+ + HCO_3$

(2) $H_2CO_3 \rightleftharpoons H + HCO_3$

(3) $H_2O \rightleftharpoons H + OH$

(4) $HCO_3 + H_2O \rightleftharpoons H_2CO_3 + OH$

Now, suppose that CO_2 is added to a mixture of bicarbonate and CO_2, $H_2\ CO_3$ is formed and this increases the concentration of HCO_3 by dissociation. The result will be an increase of non-dissociated $NaHCO_3$. Now, if a solution of 1 Kg. of sodium bicarbonate in 100 litres of H_2O is taken and is allowed to attain equilibrium with an unlimited atmosphere containing 1 gm. of CO_2 per litre. And then add HCl, there will not be much change from the usual pH. The cause of this is that when HCl is added it reacts to form NaCl and more CO_2. The latter being in excess escapes to the atmosphere and the total amount of acid is what it was before. Thus only the concentration of the bicarbonate is diminished.

For example if HCl is introduced into a cell, it will get ionized and the free H-ions will greatly increase. But if say sodium phosphate is present then sodium chloride will be formed and phosphoric acid will be released. This is very weakly ionized and thus appriciable change in the pH will not take place. But the presence of NaCl instead of Sodium phosphate will not affect for obvious reasons. Thus Sodium chloride has no buffer action.

Determination of H-ion Concentration.

(1) *Colorimetric.*

Colorimetric measurements of H-ion concentration are based upon the behaviour of certain weak organic acids which change colour when neutralized. An acid

like Methyl red gives a rose coloured solutions, while its salts are yellow. When half neutralised both rose acid and yellow salt are present giving an orange colour. The colour changes gradually over a characteristic H-ion range.

Indicators are generally salts of very weak acid or base. The undissociated salt has one colour and the dissociated ions have another. Now an indicator dissociates to different extents in different H-ion concentrations, thus giving different colours with the indicators. Here care should be taken to use the correct indicator for a given range of H-ion concentration. For instance if a solution of HCl is taken Methyl orange is red in it. Alkali is slowly added till the colour changes to just orange. That is the solution is just alkaline to this indicator. If another sample of acid is taken it will be found to produce no colour with phenolphthalein and more alkali will have to be added to change colour to red.

Care should be taken to use only small amounts of the indicators so as not to neutralise any perceptible portion of the ions to be estimated. If we take congo red, it is a sodium salt of an acid. In its undissociated form it is red but its free acid gives blue colour. Now add a drop of this solution to a dilute solution of HCl a blue colour is given. Because in greater H-ion concentration greater quantity of the undissociated indicator gets dissociated. Now if a concentrated solution of the indicator is added in large quantity to small amount of very weak concentration of HCl the colour

remains red. Because the whole of the free HCl present has combined with a portion only of the dye and the colour of the salt left in excess masks the bluish colour of the very small amount of the free acid dye.

There are many other indicators. Neutral red is one of the valuable one for Physiological purposes. It changes colour at the neutrality of the water and changes colour just above and below the neutral point.

The single standard colorimetric method is one in which all the standard indicator is in one tube.

Double standard.—If Methyl red indicator is so used that half of the compound is placed in a solution more acid than pH 4.2 and half in more alkaline than 6.3 the orange colour observed when the 2 solutions are observed together is the same as produced in a single tube at a pH which results in the neutralization of half the indicator acid.

Double standard colour can be prepared with Bromocresol green, bromocresol purple, phenol red, etc.

How to determine.

To determine the pH of an unknown, first test small portions with a drop of each of the indicators and use the indicator which developes most nearly its mid colour.

Potentiometric measurements.

When a solid is placed in water it has a tendency to send out its molecules in water so as to form a solution. The intensity of this varies with different cases

and is known as solution pressure of the substance in question. And when a metal say copper is immersed in a solution of copper sulphate, the copper has a tendency to give off Cu^{++} ions into solution. But there are already ions of the same kind in the solution which by their Osmotic Pressure (O.P.) oppose the passage of similar ions from the metal. The force with which the metal tends to send out ions in solution is the electrolytic solution pressure and may be greater or less than the O.P. of the metallic ions in solution. It is plain that if the solution pressure is greater, the metal will become negatively charged owing to its giving off positive charges with the ions leaving it. Its potential will depend upon the difference between the solution pressure and O.P.

But any of these electrodes above will not work unless the circuit is completed by having metal at both ends of the cells. If, however, the two electrodes of the same metal in solutions of the same concentration are joined together then no E. M. F. will develop. But if the concentration of the metallic ion is different in the two solutions, the E. M. F. of the battery will be equal to the difference between the potential of the two electrodes. If we know the concentration of one solution and can measure the E. M. F. of the combination, we can know the concentration of the unknown solution by the difference.

The Hydrogen electrode is based on the above principles. If we could make an electrode of this gas and immerse it in a solution containing H-ions we can

measure the concentration of H-ions by the potential of the electrode. In practice, platinum is used as an electrode and is plated with platinum black, in order to rapidly saturate it with H. One may note that it is unnecessary to have both Hydrogen electrodes; so long as the opposing electrode is of known E. M. F. it may be of any form. Often in practice Ostwald's calomel electrode is used.

The measurement is made by a comparator or by the potentiometer method. Here a current is made to pass through a wire from a constant battery. By means of a sliding contact any fraction of the E. M. F. between two ends of the wire can be tapped off and opposed to that of the electrodes until the whole is brought to zero.

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